ANTI-ULCER AND WOUND HEALING ACTIVITIES OF *Dipteracanthus patulus (Jacq)* LEAF EXTRACTS

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MASTER OF PHARMACY

IN

PHARMACOLOGY

By

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EVALUATION SHEET

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LIST OF ABBREVIATIONS

D.patulus	-	*Dipteracanthus patulus*
CPSCEA	-	Committee for the Purpose of Control and Supervision of Experimental Animals
NSAID	-	Non steroidal anti-inflammatory drug
PUD	-	Peptic ulcer disease
TLC	-	Thin-layer chromatography
FT-IR	-	Fourier Transmitted Infra-red spectroscopy
R_f	-	Retention factors
CDH	-	Central drug house, New Delhi
UI	-	Ulcer index
CMA	-	Chorioallantoic Membrane Assay
mg	-	Milligram
µg	-	Microgram
kg	-	Kilogram
p.o	-	Per oral
cm	-	Centimetre
mm	-	Millimetre
ml	-	Millilitre
Oint	-	Ointment
W/W	-	Weight per Weight

Chapter 1

1.1 GENERAL INTRODUCTION

Medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis of medicines. Plants play a vital role in the development of human cultures around the whole world**.** Moreover, some plants consider as important source of nutrition and as a result of that plants recommended for their therapeutic values.

Today the term “Alternative Medicine” became very common in western culture, it focus on the idea of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so, most of these pills and capsules we take and use during our daily life came from plants. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics, and anti-malaria medications contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, vinca and opium poppy, respectively.

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of future studies. [1]

Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. [2]

Chapter 2

REVIEW OF LITERATURE

2.1. General

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. India has several traditional medical systems, such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. The materia medica of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India. The ancient texts like Rig Veda (4500-1600 BC) and Atharva, Veda mention the use of several plants as medicine. In addition to their natural role, plant secondary metabolites also represent a vast resource of complex molecules that are valued and exploited by man for their pharmacological and other properties (Table 2.1)

Table 2.1: Some examples of plants as source of drugs.[3]

Plants	Part used	Uses
Atropa belladonna	Whole plant	Sedative
*Adhatoda zeylanica*Medicus.	Leaf	Asthma, Cold
Aloe vera Linn.	Leaf	Wound healing
Betel piperL.	Leaf	Pimples
Cardiospermum canescens Wall.	Leaf	Dysentery
Cassia fistula Linn.	Leaf	Laxative
Erythrina indica Lam.	Leaf	Menstrual problem
Eucalyptus globules Labill.	Leaf	Body ache, Re-freshener
Euphorbia anticaram L.	Latex	Edema
Ficus bengalensis L.	Latex	Wound healing
Lawsonia inermis L.	Leaf	Heeling crack
Ocimum santum L.	Leaf	Dry cough
Phyllanthus amarus Schum.	Leaf	Jaundice
Quercus infectoria	Seed husk	Wound ,Anti-inflammatory

In India, the ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostic characters can provide a better understanding of their active principles and mode of action. However, a large number of tropical plants have not been studied in detail for their chemical constituents, pharmacological properties of the extracts, and their pharmacognostic characterization including DNA sequencing etc. In the present review focused various aspects in selected medicinal plant *Dipteracanthus patulus (Jacq)*

2.2 SELECTION OF PLANT FOR STUDIES

Dipteracanthus patulus (Jacq) commonly known as kiranti nayan is widely used in traditional medicine in India. Extracts of root, leaves, and stem of this plant are widely used in the treatment of Snake bite, wounds, scabies, anti-inflammatory, and ulcers.

2.2.1Plants preferred for present study:

Sl. No.	Botanical Name of the Plant	Family	Part selected
01	*Dipteracanthus patulus (Jacq)*	Acanthaceae	Leaves

2.2.2 MORPHOLOGY of plant

Botanical Name : *Dipteracanthus patulus*

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Genus : *Dipteracanthus*

Species : *patulus*

Family : Acanthaceae

Dipteracanthus patulus

Vernacular Names:

Tamil	**:**	Kiranti nayan
Malayalam	**:**	Velipadakkam, Thuppalampotti
English	**:**	Bell weed

2.2.3 DESCRIPTION OF PLANT:

Dipteracanthus patulus (Jacq) Undershrub, up to 50 high; branchlets 4-angled, pubescent. Leaves opposite, ovate-elliptic, 1.5 - 5 x 1.2 - 3 cm, cuneate-truncate at base, entire at margin, acute at apex, densely pubescent; 4 - 5-pairs; petiole up to 1.8 cm long. Flowers ca. 2.5 cm across, pale blue or violet, solitary, axillary, 2 - 3-flowered; bracteole spatulate-elliptic, ca. 1 cm long, obtuse at apex. Calyx - lobes 5, equal, linear-subulate to lanceolate, 03 mm long, ciliate at margin. Corolla campanulate-infundibuliform, lobe 5, subs orbicular-ovate, ca. 08 mm long. Ovary oblong-elliptic, ca. 02 mm long; 1-loculed; ovules ca. 6; style filiform, 03 mm long, white hairy; stigma subglobose. Capsules clavate – elliptic to cylindrical up to 1.8 cm long, glabrous; seeds ca. 10, orbicular, ca. 03 mm long. The plant was distributed widely in Pune, Satara,thane, Chikmagalur, Dharwar, Mysore, Idukki, Kannur, Kollam, Thiruvananthapuram, Tamil nadu in all districts.

Some vernacular names in India:

cilantanceti, cilantinayakam, cukkulam, cuntuilicceti, cuntumam, cuntuyili, icaimuti, kakapicam, kakapikanayacceti, kattunayakacceti, kattunayakam, kiranti, kiranti nayakam, kirantinayakan, kirantippuntu, nittinaviral, nittinaviralkurittan, punkiranti, punkiranticceti, putakilam, putakilanceti, turuputpam, nayakam, upu-dali, vaikkirantitacceti, vaikkirantitam.[4]

Constituents

The major phytoconstituents found in *Dipteracanthus patulus* are the Tannis, saponins, alkaloids, steroids anthroquinones, tri-terpenoids, and flavonoids. Two lignan glycosides,5,5'-dimethoxylariciresinol-9'-O- β-D-glucopyranoside and lyoniresinol–9'-O-β–D- glucopyranoside were isolated from the extracts of whole plant of *Dipteracanthus patulus.*[5]

2.3 PEPTIC ULCER:

Peptic ulcer disease is a chronic pathology that affects millions of people worldwide. It is believed that 10% of the population will develop this condition at some point in their lives. Peptic ulcers are usually classified by their anatomic location, such as gastric or duodenal ulcers, and increased gastric acid is the main cause. There is a strong association between *H. pylori* infection and duodenal ulcers. *H. pylori* causes an inflammatory response in the gastric mucosa, with increased production of cytokines and influx of neutrophils and macrophages into the gastric mucosa with release of leukotrienes (LT) and reactive oxygen species, which makes the defense of the mucosa and stimulates ulcer formation process. The disease process of peptic ulcers is multifactorial based on etiology and risk factors. Ulcers result from an imbalance between aggressive factors, including hydrochloric acid; *Helicobacter pylori* infection; excessive intake of anti-inflammatory drugs, alcohol, pepsin, and reactive oxygen species; and cytoprotective factors, which include mucus, bicarbonate, prostaglandins, blood flow, and cellular repair, as well as enzymatic and non-enzymatic antioxidants. Currently, the goals of treatment of peptic ulcer are based on pain relief, heal the ulcer and prevent recurrence of the ulcer.Thus, gastric ulcer treatment options include antacids (aluminum hydroxide and magnesium trisilicate), cytoprotective agents (sucralfate and the prostaglandin analogue misoprostol), muscarinic antagonists (pirenzepine), antimicrobial agents for eradication of *H. pylori* (amoxicillin and clarithromycin), H2 receptor antagonists (cimetidine and ranitidine), and proton pump inhibitors (omeprazole and lansoprazole). Many adverse effects are associated with the prolonged use of H2 receptor blockers and proton pump inhibitors: hypersensitivity, arrhythmia, impotence, gynecomastia, and hypomagnesemia. Moreover, some of these treatments are expensive.[6]

2.3.1 NSAID-induced ulcer:

A small but important percentage of patients have adverse gastrointestinal events associated with NSAID use that results in substantial morbidity and mortality. Risk factors for the development of NSAID-associated gastric and duodenal ulcers

include advanced age, history of previous ulcer disease, concomitant use of corticosteroids and anticoagulants, higher doses of NSAIDs, and serious systemic disorders. The concept of gastro duodenal mucosal injury has evolved from the notion of topical injury to concepts that involve multiple mechanisms.

NSAIDs initiate mucosal injury topically by their acidic properties. By diminishing the hydrophobicity of gastric mucus, endogenous gastric acid and pepsin may injure surface epithelium. Systemic effects of NSAIDs appear to play a predominant role through the decreased synthesis of mucosal prostaglandins. The precursor of prostaglandins, arachidonic acid, is catalyzed by the two cyclooxygenase isoenzymes, cyclo-oxygenase-1 and cyclo-oxygenase-2. The gene for cyclo-oxygenase-1, the housekeeping enzyme, maintains the homeostasis of organs. Cyclo-oxygenase-2, the inflammatory enzyme, is inducible. Although NSAIDs can inhibit both pathways, only the gene for cyclo-oxygenase-2 contains a corticosteroid-responsive repressor element. Literature suggests that the anti-inflammatory properties of NSAIDs are mediated through inhibition of cyclo-oxgenase-2, and adverse effects, such as gastric and duodenal ulceration, occur as a result of effects on the constitutively expressed cyclo-oxygenase-1. H. pylori is prevalent among 22–63% of patients taking NSAIDs. Most studies do not show a significant difference in H. pylori prevalence between NSAID users and nonusers. Gastritis in patients on NSAID therapy appears to be related to underlying H. pylori rather than drug use. The lower incidence of H. pylori among patients with gastric ulcers than those with duodenal ulcers is presumably the result of NSAID use. NSAIDs are more likely to cause gastric than duodenal ulcers. NSAID appear to cause ulcers by a mechanism independent of H. pylori based on the inhibition of prostaglandin synthesis.

2.3.2 GASTRINOMA (Zollinger-Ellison Syndrome):

The classic triad of Zollinger-Ellison syndrome involves peptic ulcers in unusual locations (i.e., the jejunum), massive gastric acid hypersecretion, and a gastrin-producing islet cell tumor of the pancreas (gastrinoma).

2.3.3 ALCOHOL AND DIET-INDUCED ULCER:

Although alcohol has been shown to induce damage to the gastric mucosa, it seems to be related to the absolute ethanol administered (200 proof). Pure ethanol is lipid soluble and results in frank, acute mucosal damage. Because most humans do not drink absolute ethanol, it is unlikely there is mucosal injury at ethanol concentrations of less than 10% (20 proof). Ethanol at low concentrations (5%) may modestly stimulate gastric acid secretions; higher concentrations diminish acid secretion. Though physiologically interesting, this has no direct link to ulcerogenic or therapy. Some types of food and beverages are reported to cause dyspepsia. There is no convincing evidence that indicates any specific diet causes ulcer disease. Epidemiologic studies have failed to reveal a correlation between caffeinated, decaffeinated, or cola-type beverages, beer, or milk with an increased risk of ulcer disease. Dietary alteration, other than avoidance of pain-causing foods, is unnecessary in ulcer patients.[7]

2.3.4 STRESS INDUCED ULCER:

Stress-related mucosal damage (SRMD) is the broad term used to describe the spectrum of pathology attributed to the acute, erosive, inflammatory insult to the upper gastrointestinal tract associated with critical illness. SRMD represents a continuum from asymptomatic superficial lesions found incidentally during endoscopy, occult gastrointestinal bleeding causing anemia, overt gastrointestinal bleeding and clinically significant gastrointestinal bleeding.[8]

Stress ulceration was first described in 1969 when focal lesions in the mucosa of the gastric fundus were reported during post-mortem examinations in 7 (out of 150) critically ill patients.[9]

2.4 WOUND HEALING:

wound healing is a complex and dynamic process, with the wound environment changing with the shifting health status of an individual. Knowledge of the physiology of the normal wound healing trajectory through the phases of hemostasis, inflammation, granulation and maturation provides a framework for understanding the basic principles of wound healing. Through this understanding, the health care professional can develop the skills required to care for a wound and the patient can be helped with the complex task of tissue repair.

2.4.1 PHASES OFWOUND HEALING:

Wounds also undergo 4 basic phases of healing

These are:

- Hemostasis,
- Inflammation,
- Proliferation (also known as granulation and contraction), and
- Remodelling (also known as maturation).

2.4.1 HEMOSTASIS:

Once the source of damage to a house has been removed and before work can start, utility workers must cap damaged gas or water lines. So, too in wound healing must damage blood vessels be sealed. In wound healing, the platelets are the cells that act as utility workers sealing off the damaged blood vessels. The blood vessels themselves constrict in response to injury, but this spasm ultimately relaxes. The platelets secrete vasoconstrictive substances to aid this process, but their prime role is to form a stable clot sealing the damaged vessel. Under the influence of ADP (adenosine diphosphate) leaking from damaged tissues.

2.4.2 INFLAMMATION:

Clinically, inflammation (the second stage of wound healing) presents as erythema, swelling and warmth often associated with pain, the classic “rubor et tumor cum calore et dolore.” This stage usually lasts up to 4 days post injury. In the damaged

house analogy, once the utilities are capped the second job is to clean up the debris. This is a job for unskilled labourers. In a wound, these unskilled labourers are the neutrophils (polymorphonucleocytes). The inflammatory response causes the blood vessels to become leaky, releasing plasma and neutrophils into the surrounding tissue. The neutrophils phagocytose debris and microorganisms and provide the first line of defence against infection.

2.4.3 PROLIFERATION:

The proliferation phase starts approximately 4 days after wounding and usually lasts until day 21 in acute wounds, depending on the size of the wound and the health of the patient. It is characterized by angiogenesis, collagen deposition, granulation tissue formation, wound contraction and epithelialization. Clinically, proliferation is observed by the presence of pebbled red tissue or collagen in the wound base and involves replacement of dermal tissues and sometimes subdermal tissues in deeper wounds, as well as contraction of the wound.

2.4.4 REMODELLING:

Once the basic structure of the house is completed, interior finishing may begin. Similarly, in wound repair, the healing process involves remodelling and realignment of the collagen tissue to produce greater tensile strength. In addition, cell and capillary density decrease. The main cells involved in this process are the fibroblasts. Remodelling can take up to 2 years after wounding.[10]

2.5 Review of various literature:

Saroja, et al., 2009wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide. Community-based epidemiological study of wounds in India revealed the prevalence of acute and chronic wounds as 10.55 and 4.48 per 1000 population respectively. Healing of chronic lower extremity wounds is a global problem. Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many traditional practitioners across the world particularly in countries like India and China with age old traditional practices have valuable information of many lesser-known either to unknown wild plants used for treating wounds and burns Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models but the potential of most of the plants remain unexplored. *Dipteracanthus patulus* (Jacq.)Nees. (Syn. *Ruellia patula Jacq*).(Acanthaceae) commonly known as Kiranthinayagam or Kayappacchilai in Tamil is a medicinal herb traditionally used in the treatment of. Wounds in the rural areas. The leaves are ground into a paste and applied on fresh wounds. The plant is commonly distributed on wastelands in Tamil Nadu. The leaves are used for treating itches, insect bites, paranychia, venereal diseases, sores, tumours, rheumatic complaints and eye diseases . Pharmacological and phytochemical studies indicated that it is a cardiotonic.[11]

Yadav, Sanjay, et al., 2012 to screen and evaluate the anti-inflammatory activity of methanolic and aqueous extracts of root, leaves, and stem of *Dipteracanthus patulus* (Jacq.) Nees in animal models to support its traditional uses. The anti-inflammatory activity using carrageenan was examined. Acute paw edema was induced by injecting 0.1 mL of 1 % (w/v) carrageenan solution, prepared in normal saline in sub-plantar region of the left hind paw of the rat. Measurements were taken at 0, 1, 2, 3 & 4 hours after the administration of the carrageenan. The extract which showed best activity were further evaluated by egg white, xylene and TPA (12-O-tetradecanoylphorbol-13-acetate) induced inflammation in rat models. Methanolic extract (26.4%) and aqueous extract

(22.8%) of stem showed the best anti-inflammatory activity in carrageenan induced paw edema as well as in the other methods at a dose of 250 mg/kg body weight. The first time, confirms the significant anti-inflammatory activity potential of methanolic and aqueous extracts of stem of *Dipteracanthus patulus* on animal models.[12]

A. Manikandan, et al.,2009the antiulcer activity of 50% hydroethanolic leaf extract of *Ruellia tuberose L.* and *Dipteracanthus patulus* (Jaca) was evaluated in rats against pylorus ligated gastric ulcer model. The plant extract were administered orally at a dose of 500mg/kg and famotidine at the dose of 20 mg/kg (standard drug). Ulcer index was common parameter studied in A the ulcer model, further, the gastric pH, total and free acidity, gastric volume and antioxidant leaves such as SOD, CAT, GSH and the effect on LPO and protein were analysed. Both the extracts produced significant reduction in ulcer index, along with increase in the antioxidant enzyme and protein leaves as compared to control group and the lipid peroxidation was reduced in the treated and drug administered groups. Thus the plant extracts possess, significant antiulcer as well as antioxidant property.[13]

Mamdouh Nabil Samy, et al., 2015 the genus *Ruellia* L. is sometimes called *Dipteracanthus*; it comprises about 150 species native to tropical and temperate North and South America. In this review, the literature data on phytochemical and biological investigations of the genus *Ruellia* are compiled. The well-recognized groups of secondary metabolites were flavonoids, lignans, coumarins, alkaloids, triterpenes, sterols, phenolic glycosides, phenylethanoids, megastigmane glycosides, benzoxazinoid glucosides and others. The extract of this genus as well as pure compounds isolated from it have been demonstrated to possess multiple pharmacological activities such as wound healing, cardiovascular, anti-hyperglycemic, antioxidant, antimicrobial, antibacterial, anticancer, antinociceptive, anti-inflammatory, cytotoxic and gastroprotective activities, purgative and angiotensin-converting enzyme inhibitory effects, estrogenic and cholinergic properties and antifertility action.[14]

N. Kannikaparameswari, et al., 2013plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. Hence the study focuses on the identification and quantification Lupeol from the methanolic leaves extract of *Dipteracanthus paulus* (Jacq.) Nees. High-Performance Liquid Chromatography with Photodiode array detector (HPLC-PDA) was used to analyse the compound of interest. The quantity of lupeol was calculated from the respective peak areas according to individual standard curves. The content of the compound in the extract was 0.04mg/g dry weight (0.004%). The results of showed study confirm the presence of good percentage of Lupeol which supports the biological potency of the plant.[15]

S. Gopalakrishnan, et al., 2011*Dipteracanthus patulus (Jacq.)* Nees.*(*Syn*. Ruellia patula Jacq).*(Acanthaceae*)* is a medicinal herb traditionally used in the treatment of wounds. The methanolic extract of the leaves of *Dipteracanthus patulus* was analyzed by gas chromatography-mass spectrometry (GC-MS). Seventeen compounds were identified which included n-Hexadecanoic acid, 9,12,Octadecanoic acid (Z, Z) -, Linoleic acid ethyl ester.[16]

N. Murugaiyan, et al., 2015bell weed (*Dipteracanthus prostratus)* is a medicinal herb, traditionally used in the treatment of wounds, anti-cancer, hypoglycemic, anti-inflammatory, anti-ulcer and anti-oxidant activities. Based on the above information, the present study is planned to know the presence of various phytochemicals in the leaves of bell weed. Biochemicals like tannin, phenol, terpenoids, flavonoids, amino acid, protein, carbohydrate, phylobatannins, volatile oils, hydrolysable tannins, and glycosides are present in aqueous and ethanol extracts, whereas steroids are absent in both extracts. Gas chromatography-mass spectrometry (GC-MS) analysis showed 36 compounds viz among those dotriacontane, 2-hexadecen-1-ol,37,11,15-tetramethyl-(R-(R*, Spinacen, 2,6,10-trimethyl-14-ethylene-14-pentadecane, 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl) and stigmasterol were highly present in leaves extract. The antimicrobial activity of *Dipteracanthus prostratus* leaves extract against *Escherichia coli, Entrobacter aerogenes, Shigella flexneri* and *Vibrio cholera* in ethanol and aqueous

extracts. The ethanolic extracts of *Dipteracanthus prostrates* showed the maximum level of zone of inhibition towards the pathogenic bacteria *Vibrio cholera* and *Entrobacter aerogenes* when compared to aqueous extracts, standard and control and the aqueous extract showed the maximum level of zone of inhibition against pathogenic bacteria like *Entrobacter aerogenes* and *Shigella flexneri* when compared to ethanol extract. The results suggest that these phytochemicals and compounds may be used against pathogenic bacteria, probably it is used to delivering new drug for cure many infectious diseases.[17]

Mudiganti Ram Krishna Rao, et al., 2015*Ruellia patula*, Luffa aegyptiaca and Thunbergia alata are luxuriant and dominant growing herbs on the road sides and on waste lands. The present study deals with the comparative phytochemical analysis of the ethyl acetate, ethanol and ethanol-water mixture extracts of aerial parts of these plants. It was found that some phytochemicals were plant specific whereas some are extract specific. The absence of Amino acids, proteins, and flavonoids was detected in all extracts of all the three plants. Other phytochemicals like steroids, saponins, terpenoids, glycosides etc. were plant specific and extract specific.[18]

N. Saranya, et al., 2014*RuelliapatulaJacq* (syn: *Dipteracanthus patulus*) belongs to the family Acanthaceae, has numerous medicinal properties but is not exploited much in modern medicine. In-vitro antimicrobial activity of the *Ruelliapatulajacq* leaves extracted with Ethanol, Methanol, and Acetone was checked for *Bacillus subtilis, Escherichia coli,* and *Aspergillus niger* by disc diffusion method. Ethanol was found to be the better solvent that its extract showed more activity against *Aspergillus niger, Escherichia coli and Bacillus subtilis* respectively. Preliminary phytochemical screening of ethanolic and methanolic extracts showed positive results for alkaloids, steroids, phenols, flavonoids, tannins and terpenoids. Genomic DNA was extracted from *RuelliapatulaJacq* leaves using the standard Cetyl Trimethyl Ammonium Bromide extraction method. The DNA extracted responded well during PCR amplification and RAPD analysis with three gene-specific primers and five random Medicinal aromatic plant primers respectively. Chromatographic fingerprinting of the ethanolic extract of the

plant leaves was analyzed for compounds present in the sample using analytical type HPLC using a C-13 Column and a UV- detector of the *RuelliapatulaJacq* for the detection of the chemical constituents in the plant. Analytical type chromatogram revealed the presence of 8 compounds in the extract under scanning at 205 nm. The preparative type HPLC chromatogram revealed the presence of 2 major compounds in the extract. The collected fractions were subjected to FT-IR spectroscopy and UV-Visible spectrum analysis. FT-IR Spectrum studies on *RuelliapatulaJacq* ethanolic extract showed the possibility of harbouring secondary metabolites with higher pharmaceutical value.[19]

Akhtar, M. Farid, et al., 1992 Cardiovascular profile of *Ruellia patula* and *Ruellia Brittoniana* was carried on isolated rabbit heart. Crude extract, butanolic and aqueous layer of both plants were tested on heart rate, force of contraction and coronary flow. Digoxin was used as a controlled drug.All the fraction showed an increase in force of contraction expect n-butanolic fraction of R.brittoniana which exhibited depression in all parameters. Coronary flow and heart rate displayed nonsignificant decrease. Therefore it was concluded that the given *Ruellia* plant are of cardiotonic nature.[20]

Ahmad, Mansoor et al., 1993 two lignan glycosides identified as 5,5′-dimethoxylariciresinol-9-O-β-D-glucopyranoside (rupaside) and lyoniresinol-9′-O-β-D-glucopyranoside along with ethyl-α-D-galactopyranoside, α-and β-D-glucose, and β-D-fructose have been isolated from *Ruellia patula.* Their structures were assigned on the bases of spectral evidence. Preliminary cardiovascular screening of butanol and aqueous fractions suggested possible cardiotonic activity. However, additional studies will be required to confirm this type of activity.[21]

Manikandan, A., and D. V. A. Dosset al.,2010the present study was conducted to investigate the presence of biochemical contents, trace elements, nutritive value evaluation and determination of molecular weight of proteins by SDS-PAGE and phytochemicals detection by HPTLC in the leaves and 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). The biochemical contents,

trace elements, nutritive value were determined by different biochemical methods, trace elements presence was detected by using Atomic Absorption Spectroscopy (AAS), while the proteins and phytochemicals were detected by using SDS-PAGE and HPTLC. *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) leaves confirmed the presence of flavonoids, glycosides, phenols saponins and showed minimum amount of trace elements with moderate nutritive value. Vitamins (E, C), total phenolics, carotenoid content and nutritive value were found to be greater in the leaves of *Ruellia tuberosa* L. The protein bands obtained in the SDS-PAGE was found to be similar for both the plant leaves. Our findings suggest that leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) is endowed with antioxidant phytochemicals and moderate nutritive value could serve as a base for future drugs.[22]

Bumrela, Shrinivas B, et al.,2011the *Dipteracanthus patulus* (Jacq) nees is undershrub belonging to the family acantheaceae. Antimicrobial activity (disc diffusion method) and antioxidant activity by different in-vitro methods (DPPH, hydrogen peroxide, nitric oxide radical scavenging and reducing power) of methanolic extract of *Dipteracanthus patulus* (MEDP) was evaluated. The qualitative and quantitative estimation of β-carotene and β- sitosterol in MEDP was carried out by high performance thin layer chromatography (HPTLC). The total phenolic content of was determined by Folin-Ciocalteu method. Experimental findings indicate promising antimicrobial activity (antibacterial and antifungal) and potent antioxidant activity of MEDP. In addition, phytochemical analysis and spectral studies of MEDP were also performed. It is presumed that antimicrobial and antioxidant activity observed with MEDP may largely be attributed to the presence of major phytoconstituents (β-carotene, β-sitosterol and iridoid glycosides) and other minor components may participate as promoters.[23]

P. Senthilkumar, et al., 2013the methanol leaf extracts of *Ruellia tuberosa* showed significant antibacterial activity against *Escherichia coli, Pseudomonasaeruginosa, Klebsiella pneumonia, Bacillus subtilis, Proteus mirablis* and antifungal activity against *Aspergillussp, Mucorsp, Penicilliumsp* and *Fusarium sp*. The antibacterial potential of *Ruellia tuberose* methanol extract was tested by using Agar

welldiffusion method. The (100mg/ml) leaf extract showed maximum inhibition against *Proteus mirablis* (7mm). Further the extract showed maximum zone of inhibition against the fungus of *Aspergillus sp* (8mm). Phytochemical tests were performed and showed that the antibacterial activity of plant *Ruellia tuberosa* leaves was due to the presence of phytochemical compounds like alkaloids, tripenoid, tannins, glycosides, saponins. GC-MS analysis revealed the presence of 27 compounds.[24]

V. Nagarjuna Reddy, et al., 2013 antitumor activity of methanolic extracts of 250, 500 mg/kg of *Ruellia tuberosa* leaves was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. Acute and short-term toxicity studies were performed initially in order to ascertain the safety of methanolic extracts of *Ruellia tuberosa*. Tumour cells (1×106cells/mice) were injected into the right hind limb (thigh) of solid tumour group animals subcutaneously and the tumour is allowed to develop for 11 days and the treatment is started from 12th day for a period of 20 days. The effect of methanolic extracts of *Ruellia tuberosa* on the growth of tumor, life span of EAC bearing hosts and simultaneous alterations in the haematological profile and histopathological profile were estimated. The methanolic extracts of *Ruellia tuberosa* showed decrease in tumor size, average body weight, mean survival time thereby increasing life span of EAC tumor bearing mice. Haematological profile reverted to more or less normal levels in extracts treated mice. Histopathology has minimal effects when compared but a significant variation is seen.[25]

S. Ramadevi, et al., 2013 estimate the phytochemical profile and antimicrobial activity of medicinal plant *Ruellia patula* (L.) against human pathogenic bacteria. Medicinal plants are the effective source for the development of drug against several diseases. Nowadays, medicinal plants were used to treat most diseases among humans because of its medicinal value. In Ayurveda and Siddha, many medicinal plants have been recommended for the management of common diseases. The extraction was done by using different solvent such as ethanol, methanol and acetone by using standard procedures. The antibacterial assay was carried by using agar well method with different organisms and also antifungal activity against *Aspergillus niger*using disc

diffusion method. The ethanolic extract of *R. patula* L. (0.4 mg/mL) showed higher antibacterial activity against Gram positive bacteria and Gram negative bacteria. In the antifungal activity also ethanolic extract shows highest activity compare to other extract. From the present work, we conclude that the ethanolic extract of plant *R. patula* L. have potential of antibacterial activity and antifungal activity because of its secondary metabolites in the plant which responsible for biological activities. Due to the presence of phyto-constituent in the plant extract may control the bacterial growth either in high concentration/long durations and it may have the ability to control the human pathogenic organisms.[26]

khurram afzal, et al., 2015 *Ruellia* is a genus of flowering plants commonly known as *Ruellias* or Wild Petunias which belongs to the family Acanthaceae. It contains about 250 genera and 2500 species. Most of these are shrubs, or twining vines; some are epiphytes. Only a few species are distributed in temperate regions. They are distributed in Indonesia and Malaysia, Africa, Brazil, Central America and Pakistan. Some of these are used as medicinal plants. Many species of the genus has antinociceptive, antioxidant, analgesic, antispasmolytic, antiulcer, antidiabetic and anti-inflammatory properties. The phytochemicals constituents: glycosides, alkaloids, flavonoids and triterpenoids are present. The genus has been traditionally claimed to be used for the treatment of flu, asthma, fever, bronchitis, high blood pressure, eczema, and diabetes. The objective of this review article is to summarize all the pharmacological and phytochemical evaluations or investigations to find area of gap and endorse this genus a step towards commercial drug. Hence, further work required is to isolate and characterize the active compounds responsible for these activities in this plant and bring this genus plants to commercial health market to serve community with their potential benefits.[27]

Bo Eng Cheong, et al., 2013 antioxidant and anti-proliferative activities of Sabah *Ruellia tuberosa*. The total phenolic and flavonoid contents of the plant extracts were determined by using Folin-Ciocalteau and aluminium chloride colorimetric assays,

respectively. The antioxidant activity of the plant extracts was evaluated using DPPH free radical scavenging assay while the anti-proliferative activity was evaluated using MTT assay against the human breast cancer (MCF-7) and cervical cancer (HeLa) cell lines. The methanol leaf extract was found to possess the highest total phenolic content (82.67 ± 2.09 mg GAE/g) while the ethyl acetate leaf extract was found to possess the highest total flavonoid content (152.77 ± 4.68 mg Cat/g). The ethyl acetate leaf possessed the highest radical scavenging activity, with IC50 of 720 µg/ml. Meanwhile, the methanol stem extract showed the highest anti-proliferative activity against MCF-7 cancer cells, with IC50 of 22 µg/ml but none of the extracts exhibited strong anti-proliferative activity against the HeLa cancer cell lines. Significant correlation was found between the total phenolic/flavonoid contents with the total antioxidant activity while weak correlation was found between the total phenolic/flavonoid contents with the inhibition of MCF-7 cell proliferation. Our findings indicate that Sabah *Ruellia tuberosa* could be a potential source for natural antioxidant as well as chemo-preventive agent against breast cancer in future. Thus, further isolation and characterization of the respective bioactive compounds from the plants are necessary.[28]

M. Rajan, et al., 2012 *Ruellia tuberosa* Linn belongs to family acanthaceae is a large sized plant distributed throughout India, Srilanka and Nepal. methanolic extract of *Ruellia tuberosa linn* leaves in normal and alloxan induced diabetic rats. The Preliminary phytochemical screening shows the presence of carbohydrates, glycosides, phytosteroids, flavonoids, tannins, fixed oils & fats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p).The methanol extract of *Ruellia tuberosa linn* leaves at a dose of 100 and 200mg/kg of body weight was administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of methanol extract of *Ruellia tuberosa linn* leaves on blood glucose, serum lipid profile [total cholesterol (TC), triglycerides (TG), high density lipoprotein – cholesterol (HDL-C), low density lipoprotein – cholesterol (LDL-C), very low density lipoprotein – cholesterol (VLDL-C) and serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT), alkaline phosphatase (ALP)], were measured in

the diabetic rats. The methanol extract of Ruellia tuberosa linn leaves elicited significant reductions of blood glucose (P<0.05), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C at the dose of 200mg/kg was compared with the standard drug Glibenclamide (5gm/kg). From the above results, it is concluded that methanol extract of *Ruellia tuberosa linn* leaves possesses significant antidiabetic, antihyperlipidaemic and hepatoprotective effects in alloxan induced diabetic rats.[29]

M. Ashraful Alam, et al., 2009 the ethanol extract of *Ruellia tuberosa* L. (Acanthaceae) was evaluated for its antinociceptive and anti-inflammatory properties in experimental mice and/or rat models. In the hot-plate test, the group that received a dose of 300 mg/kg showed maximum time needed for the response against thermal stimuli (5.11 ± 0.12), which was similar to that of diclofenac sodium (5.96 ± 0.18), a well-known painkiller. The maximum possible analgesia (MPH) was 1.93 for the extract dose 300 mg/kg, while that for diclofenac was 2.29 after 60 min of administration in the hot tail-flick method. The extract at 500 and 250 mg/kg doses showed significant reduction in acetic acid-induced writhing in mice with a maximum effect of 63.21% reduction at 500 mg/kg dose, which was similar to positive control diclofenac sodium (66.98%). The extract also demonstrated significant inhibition in serotonin and egg albumin-induced hind paw edema in rats at the doses 100, 200 and 300 mg/kg (serotonin-induced edema 35.85, 46.78 and 55.18%; egg albumin-induced edema 42.96, 48.30, and 55.61% inhibition after 1-4 h). The anti-inflammatory properties exhibited by the extract were comparable to that of indomethacin at a dose of 5 mg/kg (serotonin-induced edema 53.22; egg albumin- induced edema 57.01% inhibition after 4 h).[30]

Christine O. Wangia, et al., 2016 medicinal plants play a significant role in treatment and prevention of many diseases in humans worldwide. *Ruellia* species belong to the family *Acanthaceae* and have been used widely for medicinal purposes. The objective of this study was to evaluate the comparative *In vitro* antioxidant activity of two Kenyan *Ruellia* species viz. *Ruellia lineari-bracteolata* (RLB) and *Ruelliabignoniiflora* (RBK). The plant materials were extracted with aqueous, ethyl acetate and methanol. The extracts were subjected to phytochemical screening

according to standard procedures and anti-oxidant activity determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Comparative anti-oxidant activity for methanolic, ethyl acetate and aqueous extracts of RLB and RBK exhibited IC50 values of (2.7, 29.3, 7.2 and 24.4, 237.2, 66.4 µg/ml) respectively. Among the three extracts, methanolic extract showed better activity (2.7 µg/ml) comparable to ascorbic standard (2.1 µg/ml). Between the two *Ruellia* species, RLB showed a significant difference ($p<0.05$) in anti-oxidant activity as compared to RBK extracts. Phytochemical screening showed the presence of terpenoids, saponins, flavonoids, tannins and glycosides. Flavonoids and tannins are the main phytoconstituents responsible for anti-oxidant activity. In conclusion, the potent anti-oxidant activity of these plants makes them useful in development of medicinal drugs for treatment and prevention of degenerative diseases.[31]

Al-Said, Mansoor S, et al., 1986 the effect of mastic, a concrete resinous exudate obtained from the stem of the tree *Pistacia lentiscus,* has been studied on experimentallyinduced gastric and duodenal ulcers in rats. Mastic at an oral dose of 500 mg/kg produced a significant reduction in the intensity of gastric mucosal damage induced by pyloric ligation, aspirin, phenylbutazone, reserpine and restraint + cold stress. It produced a significant decrease of free acidity in 6-h pylorus-ligated rats and a marked cytoprotective effect against 50% ethanol in rats which could be reversed by prior treatment with indomethacin. The protective effect was not seen when it was given intraperitoneally in phenylbutazone and restraint + cold stress models. The reduction in the intensity of ulceration in cysteamine-induced duodenal ulcers was not found to be statistically significant in mastic-pretreated rats. The results suggest that mild antisecretory and a localized adaptive cytoprotectant action may be responsible for its anti-ulcer activity. These observations support the results of an earlier study on the clinical effectiveness of mastic in the therapy of duodenal ulcer.[32]

Abdulla, M. A, et al., 2010 the anti-ulcerogenic activity of ethanol extract of *Centella asiatica* against ethanol-induced gastric mucosal injury in rats. Five groups of adult *Sprague Dawley* rats were orally pre-treated respectively with carboxymethyl cellulose (CMC) solution (ulcer control group), Omeprazole 20 mg/kg (reference group), and 100, 200 and 400 mg/kg *C. asiatica* leaf extract in CMC solution (experimental groups), one hour before oral administration of absolute ethanol to generate gastric mucosal injury. Rats were sacrificed and the ulcer areas of the gastric walls were determined. Grossly, the ulcer control group exhibited severe mucosal injury, whereas pre-treatment with *C. asiatica* leaf extract exhibited significant protection of gastric mucosal injury. Histological studies revealed that ulcer control group exhibited severe damage of gastric mucosa, along with edema and leucocytes infiltration of submucosal layer compared to rats pre-treated with *C. asiatica* leaf extract which showed gastric mucosal protection, reduction or absence of edema and leucocytes infiltration of submucosal layer. Acute toxicity study did not manifest any toxicological signs in rats. The finding suggests that *C. asiatica* leaf extract promotes ulcer protection as ascertained grossly and histologically compared to the ulcer control group.[33]

Shukla, A, et al., 1999 the healing of an open wound depends on two major phenomena: (1) epithelialization, ie replication and movement of epithelial cells; and (2) formation and contraction of granulation tissue. This second phenomenon is essential in order to maintain tissue continuity, to reduce the size of the wound and to produce a permanent scar. Pathological phenomena resulting in fibrosis (eg pulmonary fibrosis, kidney fibrosis, liver cirrhosis and stroma reaction to epithelial tumours) are characterized by a more permanent presence of a connective tissue bearing features of granulation tissue that results in excessive extracellular matrix deposition and in production of soft tissue deformation. In most cases, wound healing ends by scar formation, ie permanent deposition of connective tissue characterized by the presence of fibroblasts, extracellular matrix components, among which collagen is the most important, and small vessels. In chronic pathological changes, such as hypertrophic scars and fibrotic lesions, an excess of extracellular matrix deposition may continue for several years.[34]

Gabbiani. G, et al., 2003 the preliminary wound healing activity of *Portulaca oleracea* was studied using *Mus musculus* JVI-1. For this purpose fresh homogenized crude aerial parts of *Portulaca oleracea* were applied topically on the excision wound surface as single and two doses in different amounts. Wound contraction and tensile strength measurements were used to evaluate the effect of *Portulaca oleracea* on wound healing. The results obtained indicated that *Portulaca oleracea* accelerates the wound healing process by decreasing the surface area of the wound and increasing the tensile strength. The greatest contraction was obtained at a single dose of 50 mg and the second greatest by two doses of 25 mg. Measurements of tensile strength and healed area were in agreement.[35]

Chapter 3
AIM AND OBJECTIVES

Peptic ulcer disease (PUD) is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries. Peptic ulcer occurs due to an imbalance between the aggressive and the defensive factors.

The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, block apoptosis and stimulate epithelial cell proliferation for effective healing. Anti-secretory drugs such as proton pump inhibitors (omeprazole, lansoprazole, etc.) and histamine H2-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion. These medicines are considered safer because of the natural ingredients with no side effects.

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated

A wound is a disruption in the continuity of cell. It is an intricate process in which the skin repairs .

The need of present study reveals about the anti-ulcer and wound healing activities on animal models.

The objectives of the present study are:

1. Collection and authentication of plant and the plant parts.
2. Extraction of plant materials with ethanol and chloroform.
3. Carrying out preliminary phytochemical screening.
4. Carrying out TLC analysis for both plant extracts.
5. To evaluate the drug and excipient interactions by FT-IR spectral studies.
6. To evaluate the following Pharmacological activities of leaf extracts of the selected plant using the various standard experimental models.
    i. Anti-Ulcer studies.
    ii. Wound healing activity.

Chapter 4

MATERIALS AND METHODS

4.1Collection and authentication of plant and the plant parts

The plant materials used in this study were leaves of *Dipteracanthus patulus (Jacq)*. (Family - Acanthaceae) is collected from the sengundrapuram, (Virudhunagar Dist, Tamil Nadu, India.). The plant was authenticated by Dr.Stephen, Department of Botany, American College, Madurai, India.

4.2 Animals used

Adult male albino rats (150 - 200g) were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPSCEA).

4.3 Preparation of Ethanol and Chloroform extracts of *D.patulus (Jacq)* Leaf.

D.patulus Leaf collected was dried at room temperature under shade 15 days and coarsely powdered. The powdered materials were extracted with ethanol and chloroform. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at -4ºC until ready for use. The yield of the extract was 66.42 % (w/w) and 33.68 % (w/w). In each experiment, the extract was diluted with water to desired concentration.

4.4 Materials used for the studies:

4.4.1 Drugs used

Ranitidine	-	Aciloc injection (Cadila)
Neomycin	-	Neos (Universal)
Diclofenac sodium	-	Cofenac (Cipla)
Saline	-	DNS (Baxter)

4.4.2 Chemicals used

Ethanol	-	CDH (central drug house, New Delhi)
Chloroform	-	CDH (central drug house, New Delhi)
Silica gel (TLC grade)	-	CDH (central drug house, New Delhi)

4.5 Methodology [36-37]

4.5.1 Preliminary Phytochemical Screening for *D.patulus (Jacq)*.

4.5.1.1 Test for Carbohydrate

4ml of the extract was dissolved separately in 4 ml of d .H_2O and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrate.

i) Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated H $_2$S0 $_4$ was added along the sides of the test tubes. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrate.

ii) **Fehling's test**

The filtrate was treated with 1 ml of Fehling, s solution A and B and heated on the water bath. A reddish precipitate was obtained show the presence of carbohydrate.

4.5.1.2 Test for proteins and free amino acid

3ml of extract was dissolved in few ml of distilled water and treated with following reagents

i) **Million's Reagent:** - Appearance of red color shows the presence of proteins and free amino acids.

ii) **Ninhydrin Reagent**: - Appearance of purple color shows the presence of proteins and free amino acids.

iii) **Biuret test**: - Equal volume of 5% sodium hydroxide solution and 1% copper sulphate solution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.

4.5.1.3 Test for phenolic compounds

3ml of extract was taken in distilled water and test for the presence of phenolic compounds was carried out with dilute ferric chloride solution (5%w/v) - Appearance of violet color indicates the presence of phenolic compounds.

4.5.1.4 Test for flavonoids

i) Aqueous NaOH solution

3ml of extract, dissolved in aqueous sodium hydroxide. Appearance of yellow color indicates the presence of flavonoids.

ii) Conc. Sulphuric acid

2ml of extract concentrated sulphuric acid was added. Appearance of Yellow orange color indicates the presence of flavonoids.

4.5.1.5 Test for Alkaloids

Wagner test:

Added 2ml filtrate of extract with 1% HCl and applied steam. 1ml of the solution added with 6 drops of Wagner's reagent. Appearance Brownish-red precipitate indicates the presence of alkaloids.

4.5.1.6 Test for Tannin.

Braemer's test

10% alcoholic ferric chloride is added to 2 ml of extract. An appearance of Dark blue coloration of the solution indicates the presence of tannin.

4.5.1.7 Test for reducing sugar.

Fehling test

Added 25ml of diluted sulphuric acid (H_2 SO_4) to 5ml of extract in a test tube and boil for 15mins. Then cool it and neutralize with 10% sodium hydroxide to pH 7 and 5ml of Fehling solution. An appearance of Brick red precipitate indicates the presence of reducing sugar.

4.5.1.8 Test for Glycosides

(a) **Legal test**: Dissolved the extract in pyridine and added sodium nitroprusside solution to make it alkaline. The formation of pink red to red color shows the presence of glycosides.

(b) **Baljet test:** To 1 ml of the test 50% methanolic extract added 1 ml sodium picrate solution and the change yellow to orange color reveals the presence of glycosides.

4.6 THIN LAYER CHROMATOGRAPHY

Thin layer chromatography is so widely used that is has become an essential technique for analyst and research workers. TLC is the almost universal analytical technique in chemical analysis for organic and inorganic matter.

TLC is a simple rapid method carried out using thin layer of adsorbents on plates. TLC not only combines the advantage of paper and column chromatography but in certain aspects it is found to be superior to either method.

TLC is an important tool in the separation, identification and estimation of different classes of natural products. When a mixture containing different components is made to ascend in a TLC plate with the help of a solvent which act as a mobile phase, there will be a preferential adsorption of different components at different places on the plate. The result is the separation of components.

4.6.1Preparation of TLC Plate:

80 gm of silica gel G was weighed and shaken to a homogenous suspension with 85 ml of distilled water for 90 sec. This suspension was poured in TLC applicator which was adjusted to 0.25 mm thickness 20 carriers' transparence of layer disappeared. The plates were dried in hot air oven at 110 c for 30 minutes (activation). The plates were then stored in a dry atmosphere and used whenever required.

4.6.2 Application of extracts for separation:

The various diluted extracts spotted on a TLC plate 2 cm above its bottom using capillary tube. Most solution for application was between 0.1-1 % strength. The starting point was equally sized as far possible and spots had diameter ranging from 2-5 mm.

Mobile phase :

Chloroform: Ethanol 9 : 1

Detecting agent: Iodine

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

4.7 Characterization of Phytoconstituents using spectroscopy techniques:

FT-IR stands for Fourier Transform Infra Red, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample.

All the separated compounds from ethanol and chloroform extract of *D.patulus* leaf extract was characterized by FTIR spectroscopy technique.

4.8 ANTI-ULCER ACTIVITY [38]

4.8.1 ASPIRIN INDUCED ULCER MODEL

Animals were divided into four groups of four animals each. The dosage of drugs was administered by following

GROUP I	:	Control (Tween 80, 5mg/kg) orally
GROUP II	:	Standard Ranitidine (30 mg/kg) orally
GROUP III	:	Ethanolic extract of *D.patulus* (100 mg/kg) orally.
GROUP IV	:	Chloroform extract of *D.patulus* (100 mg/kg) orally.

The gastric ulcer was induced in each rat by administrating aspirin 500 mg/kg orally. After 45 min ethanolic and chloroform extract of *D.patulus* and other drugs were administered for seven days. The animals were sacrificed and stomach was excised and cut along the greater curvature, rinsed gently with saline to remove the gastric content and blood clots and the ulcer index was calculated.

4.8.2 ETHANOL-INDUCED ULCER MODEL

The animals were divided into four groups. The gastric ulcers were induced in rats by administrating absolute ethanol (99%) (1 ml/200 gm) orally after 45 min ethanolic and chloroform extract of *D.patulus* and other drugs were administered for seven days. The animals were sacrificed under anaesthetized conditions, and the stomach was dissected out and ulcer index was calculated.

GROUP I	:	Control (Tween 80, 5mg/kg) orally.
GROUP II	:	Standard Ranitidine (30 mg/kg) orally.
GROUP III	:	Ethanolic extract of *D.patulus* (100 mg/kg) orally.
GROUP IV	:	Chloroform extract of *D.patulus* (100 mg/kg) orally.

Ulcer index (UI) was calculated by the following formula

$$\text{Ulcer index} = 10/x$$

X= Total mucosal area / total ulcerated area

The percentage inhibition was calculated by the following formula

$$\% \text{ inhibition} = \frac{\text{UI control} - \text{UI treated}}{\text{UI control}} \times 100$$

4.9 WOUND HEALING ACTIVITY [39]

4.9.1 IN VIVO EXCISION WOUND MODEL:

The animals were numbered, weighed and then divided into four groups with five animals in each as follows:

GROUP I	:	control, simple ointment.
GROUP II	:	2%, w/w, Neomycin ointment.
GROUP III	:	20%, w/w, Ethanolic extract of *D.patulus*.
GROUP IV	:	20%, w/w, Chloroform extract of *D.patulus*.

Hairs were removed from the dorsal thoracic central region of anaesthetised mice. The mice were depilated on the back. One excision wound was inflicted by cutting away a 300 mm^2 full thickness of skin from a predetermined area; the wound was left undressed to the open environment.

Percentage of wound contraction,

$$\frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

4.9.2 IN VITRO CHORIOALLANTOIC MEMBRANE MODEL [40-41]

Embryonated chicken eggs (9 days old) were selected then divided into four groups and a small window, (1cm2) was made in the shell follows

GROUP I	:	Negative Control saline.
GROUP II	:	Positive Control Diclofenac sodium (50 µg/ml)
GROUP III	:	Ethanolic extract of *D.patulus*. (500 µg/ml)
GROUP IV	:	Chloroform extract of *D.patulus*. (500 µg/ml)

Whatman No. 1 filter paper was Small disks were generated using a standard 5 mm hole puncher, sterilized by autoclaving and stored for further use. The pre-sterilized filter disks were saturated with different concentrations of the crude extract, from 500 µg/ml, and the control solutions. Diclofenac sodium 50 µg/ml and sterile saline were used as positive and negative controls respectively. Eggshell window was closed and incubated at 37^0 C for 72 hrs. The window was then opened and the growth of new capillary blood vessels were observed and finally compared with the positive and negative control.

Chapter 5

EXPERIMENTAL RESULTS

5.1 Preliminary phytochemical screening

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents in ethanolic and chloroform extract of *D.patulus(Jacq)* Presented in Table no.1.

Table no: 1

Preliminary phytochemical screening of the ethanolic and chloroform extract of *D.patulus (Jacq)*.

S. No.	Constituents	Ethanol extract of *D.patulus*	chloroform extract of *D.patulus*
1.	Carbohydrate	-	-
2.	Proteins & amino acids	-	-
3.	Flavonoids	+	+
4.	Alkaloids	+	+
5.	Tannin	+	+
6.	Glycosides	+	-
7.	Anthroquinone	+	+
8.	Tri-terpenoids	+	+
9.	Saponins	+	-
10.	Phenol	-	+
11.	Steroids	+	+

Where, + = Presence, − = Absence

5.2 R_f values of Ethanolic and Chloroform extracts of *D.patulus(Jacq).*

Table 2: R_f values

S.no	Extracts	R_f value
1.	Ethanol extract of *D.patulus*	0.96
2.	Chloroform extract of *D.patulus*	0.92

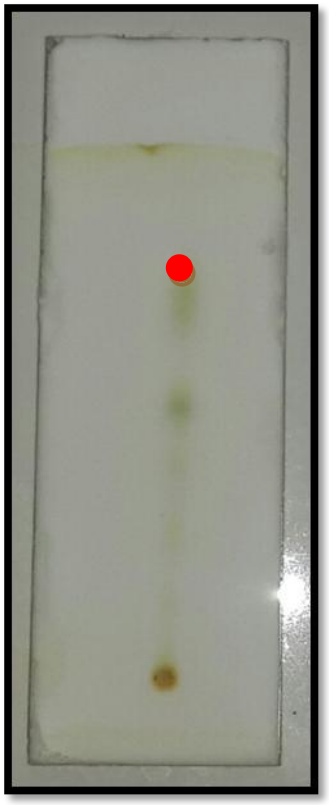

Fig no 1: Ethanolic extract of *D.patulus (Jacq)* in TLC plate.

R_f value = $\frac{4.8}{5}$ = 0.96

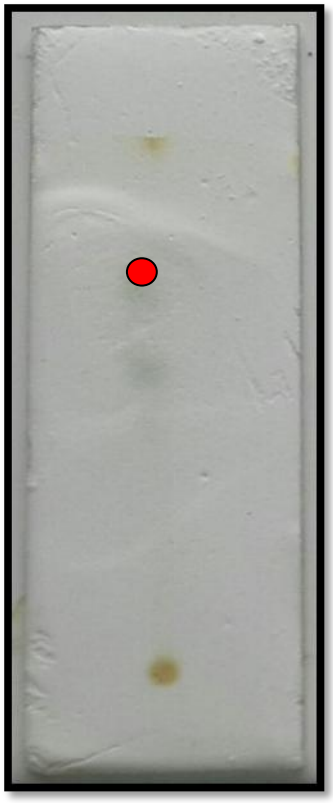

Fig no 2: Chloroform extract of *D.patulus (Jacq)* in TLC plate.

R_f value = $\frac{4.6}{5}$ = 0.92

5.3 FT-IR studies of separated compound

The IR spectrum showed an absorption of Ethanolic extract at 3020, 2927, 2148, 2364 cm^{-1} indicating the presence of C-H stretching of CH3, C-H stretching of CH2, C=C stretching, and chloroform extract at 1045,1087,1381,1649 cm^{-1} indicating the presence of C-H stretching, C-O bending, C=C stretching, The reports are showed in **Fig no- 24 & 25**

5.4 ANTI-ULCER ACTIVITY

5.4.1 Effect of Aspirin-Induced Ulcer

The results obtained in the experimental model of aspirin-induced gastric ulceration in rats are presented in **Table 3**. The ethanolic and chloroform extract was found to possess remarkable ulcer-protective properties at100 mg/kg. The effect of ulcer protection of ethanol extract of *D.patulus(Jacq)* (45.24%) was observed at 100 mg/kg and chloroform extract of *D.patulus(Jacq)* (38.24%) was observed at 100mg/kg, whereas the standard drug ranitidine gave (69.65%) of ulcer protection.

5.4.2 Effect of Ethanol-Induced Ulcer

The results obtained in the experimental model of ethanol -induced gastric ulceration in rats is summarized in **Table 4**. The ethanolic and chloroform extract was found to possess remarkable ulcer-protective properties at 100 mg/kg. The effect of ulcer protection of ethanol extract of *D.patulus(Jacq)* (52.69%) was observed at 100 mg/kg and chloroform extract of *D.patulus(Jacq)* (48.34%) was observed at 100mg/kg, whereas the standard drug ranitidine gave (73.21%) of ulcer protection.

5.5 WOUND HEALING ACTIVITY

IN VIVO MODEL

5.5.1 Excision wound model

The results of excision wound model are shown in **table 5**. The ethanolic and chloroform extract showed significant wound healing activity as compared to control and standard in excision wound model. It is observed that the wound contracting ability of the 20% (w/w) extract ointment treated groups showed significant wound healing from the fourth day onwards. The wound closure time was lesser, as well as the percentage of wound contraction was more with the 20% (w/w) extract ointment treated group.

Significant increase in the rate of wound contraction has been observed on day 14 (99. 01 %) on the 20 % (w/w) of chloroform extract treated animal. The post wounding was observed in 20 % (w/w) extract ointment treated animals and Neomycin ointment treated animals 100 % wound closure was observed on 16^{th} day.

IN VITRO MODEL

5.5.2 Chorioallantoic membrane model (CAM)

The result was tabulated by counting the number of blood vessels in various treatments. Ethanolic and chloroform extract, positive control promoted an increase in number of blood vessels compared to negative control saline.

Treated 500 µg/ml Ethanolic and chloroform extract group and 50 µg/ml Diclofenac sodium positive control treated group was observed formation of new blood vessels. **(Fig no 20, 21, & 22)**

The negative control does not show the formation of blood vessels. **(Fig no 23)**

ANTI-ULCER ACTIVITY

Aspirin-Induced Ulcer model

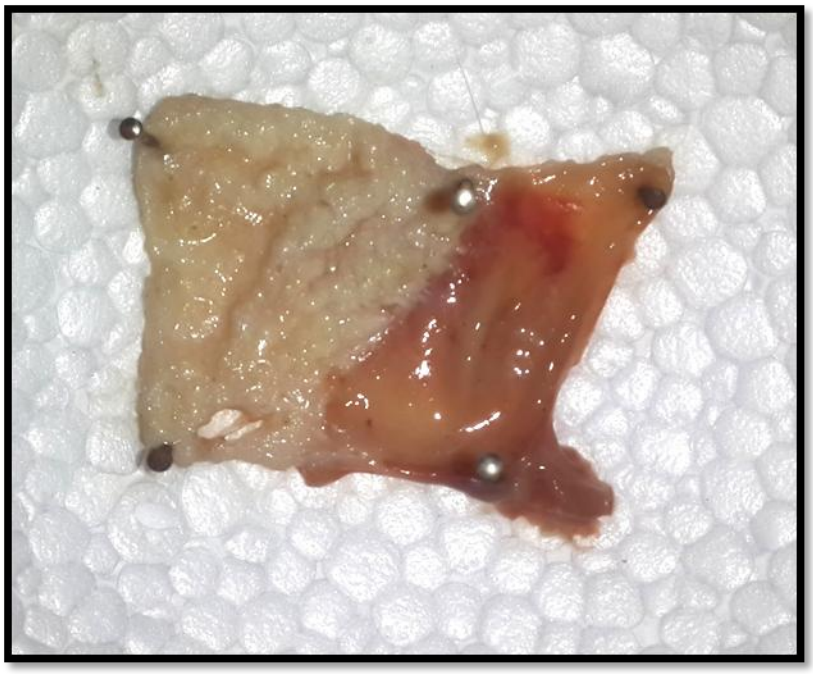

Fig-3 control- Aspirin induced ulcer model.

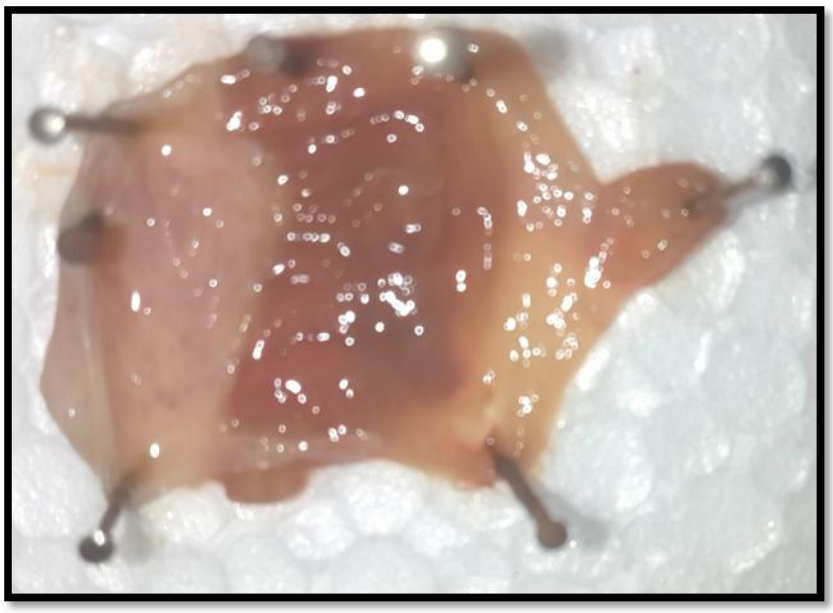

Fig-4 STD Ranitidine- treated Aspirin induced ulcer model.

Fig- 5 Ethanolic extract of *D.patulus(Jacq)* 100mg/kg treated- Aspirin induced ulcer model.

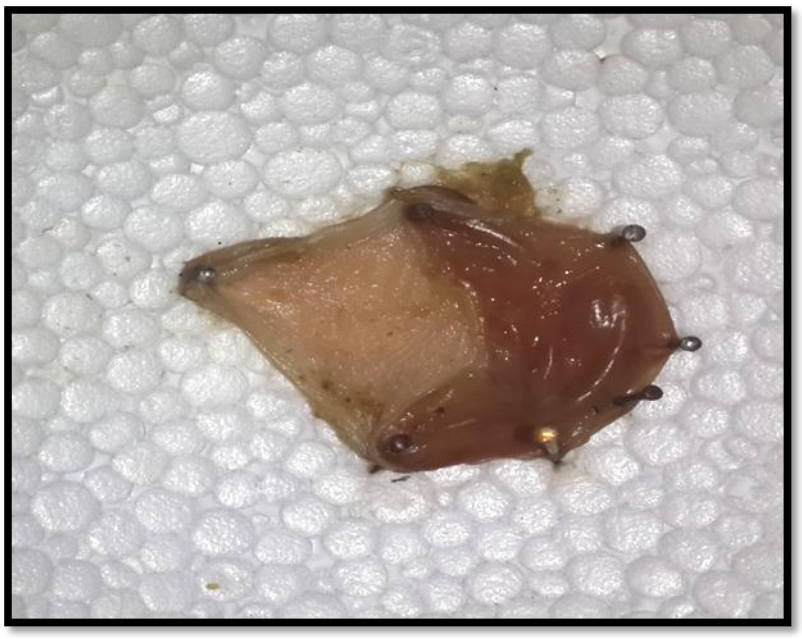

Fig- 6 Chloroform extract of *D.patulus(Jacq)* 100mg/kg treated- Aspirin induced ulcer model.

Ethanol Induced Ulcer model

Fig-7 control-Ethanol induced ulcer model.

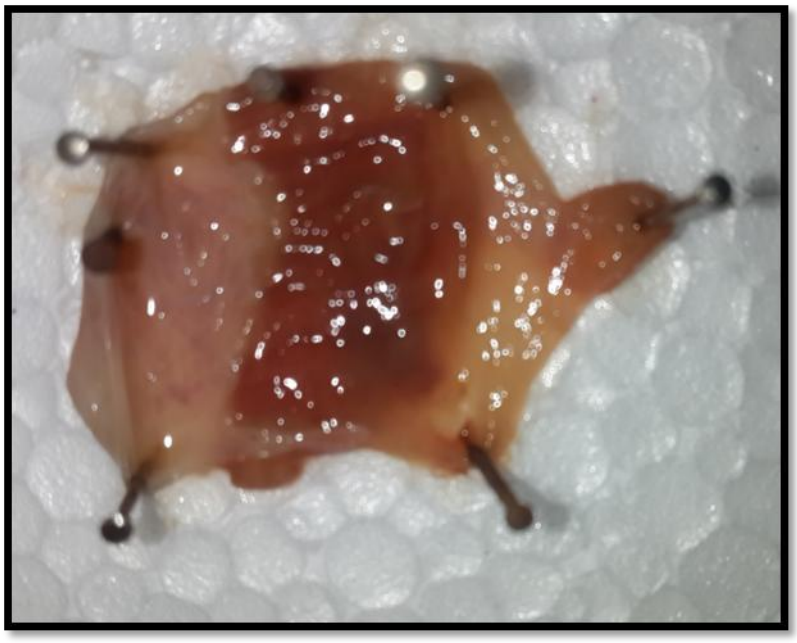

Fig-8 STD Ranitidine treated- Ethanol induced ulcer model.

Fig- 9 Ethanolic extract of *D.patulus (Jacq)* 100mg/kg treated- Ethanol induced ulcer model.

Fig- 10 Chloroform extract of *D.patulus(Jacq)* 100mg/kg treated- ethanol induced ulcer model.

WOUND HEALING ACTIVITY

Excision wound model

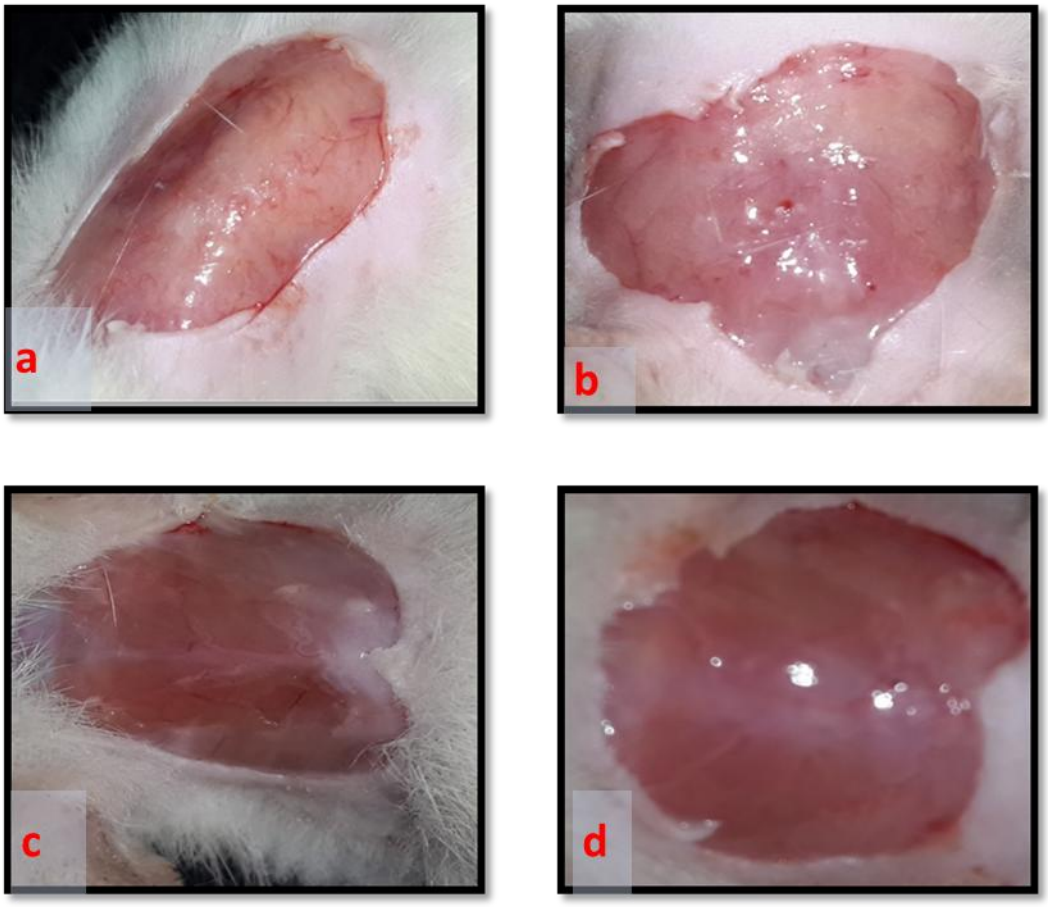

FIG NO 11: Excision wound model on initial day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

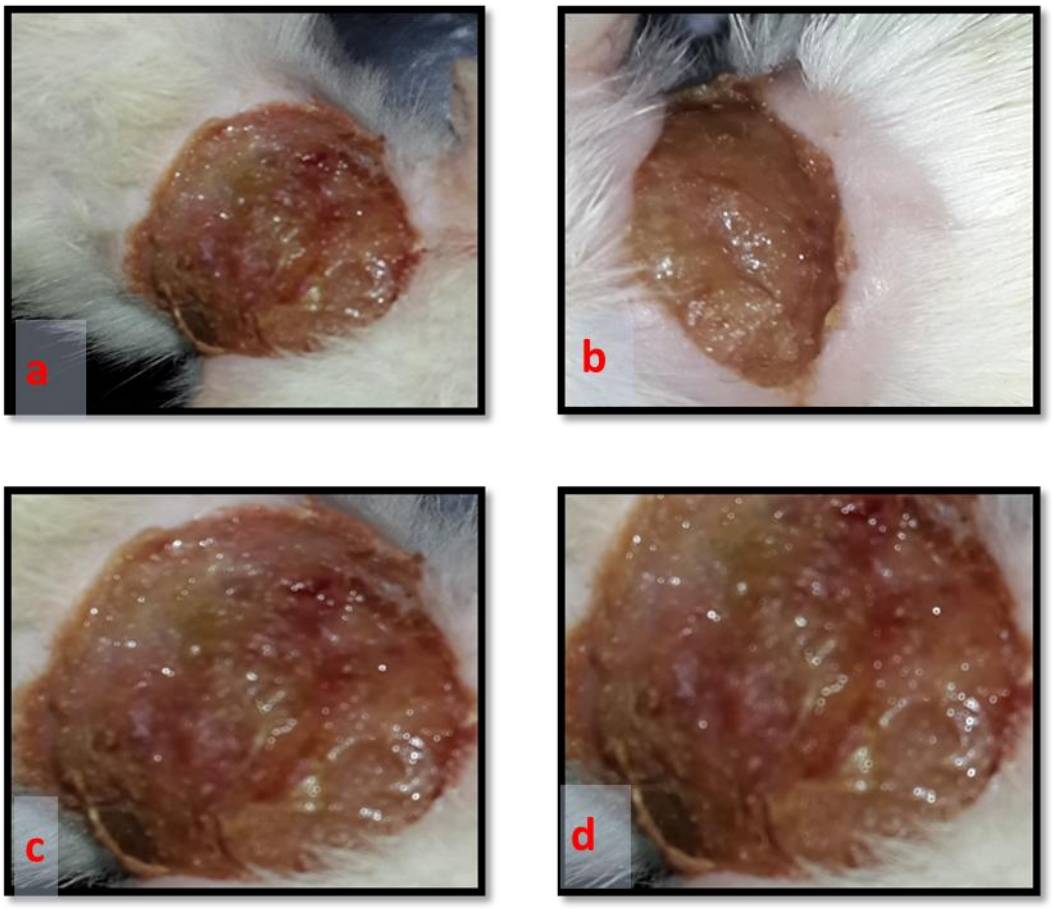

FIG NO 12: Excision wound model on 2nd day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

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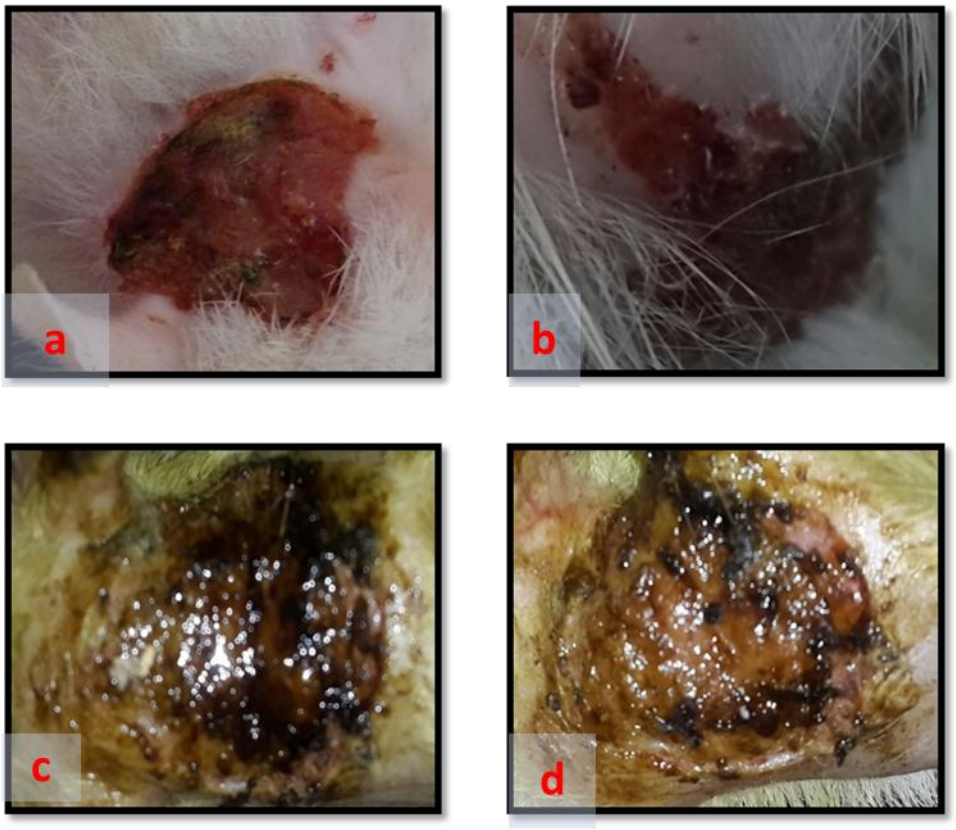


FIG NO 13: Excision wound model on 4th day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

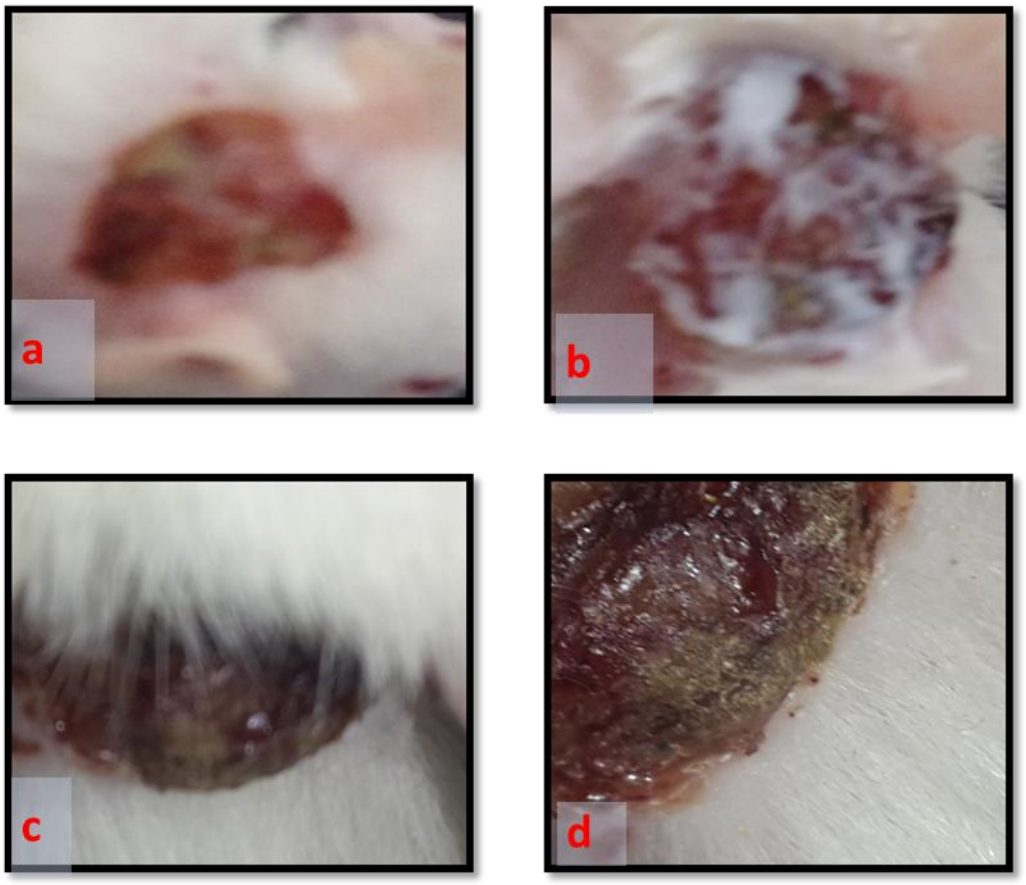


FIG NO 14: Excision wound model on 6th day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

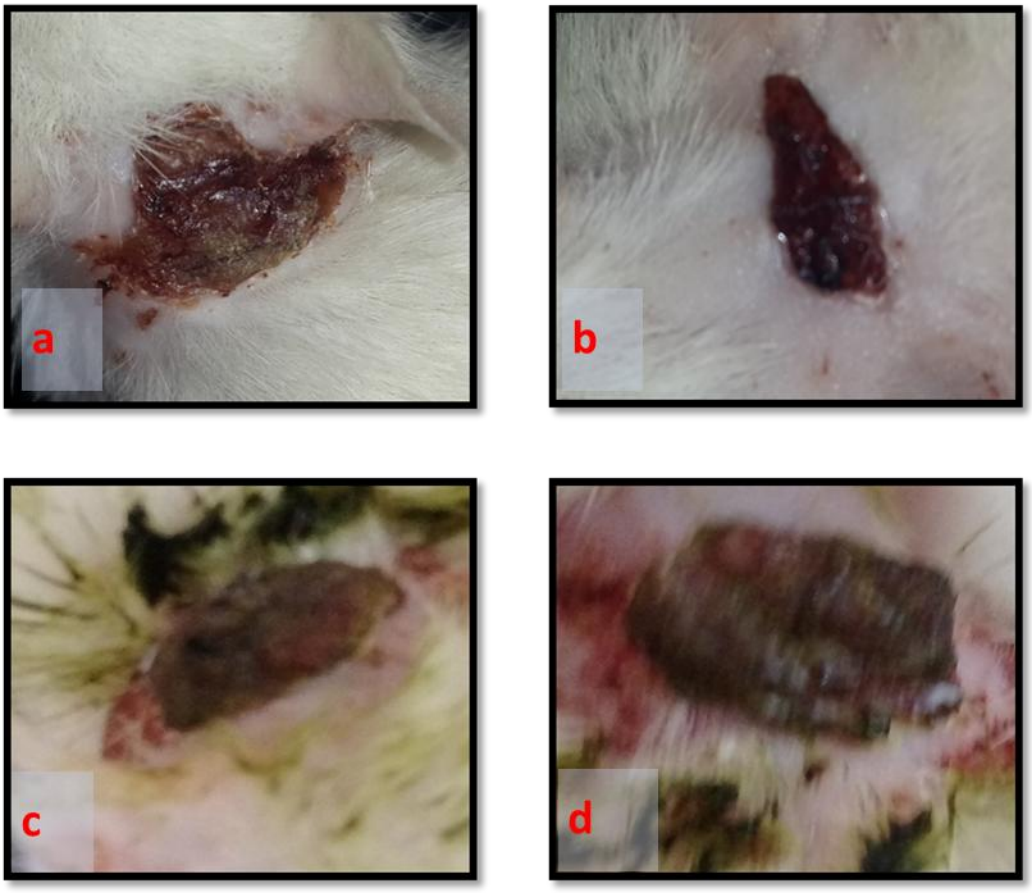


FIG NO 15: Excision wound model on 8th day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

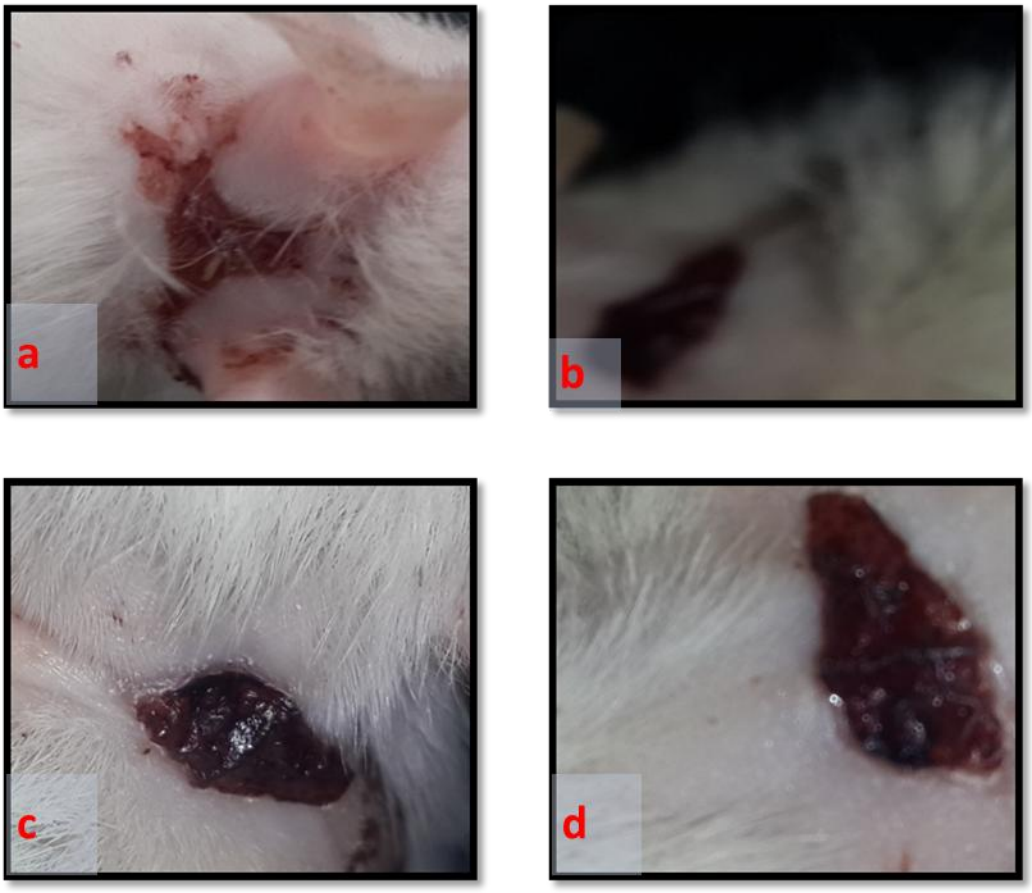


FIG NO 16: Excision wound model on 10th day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

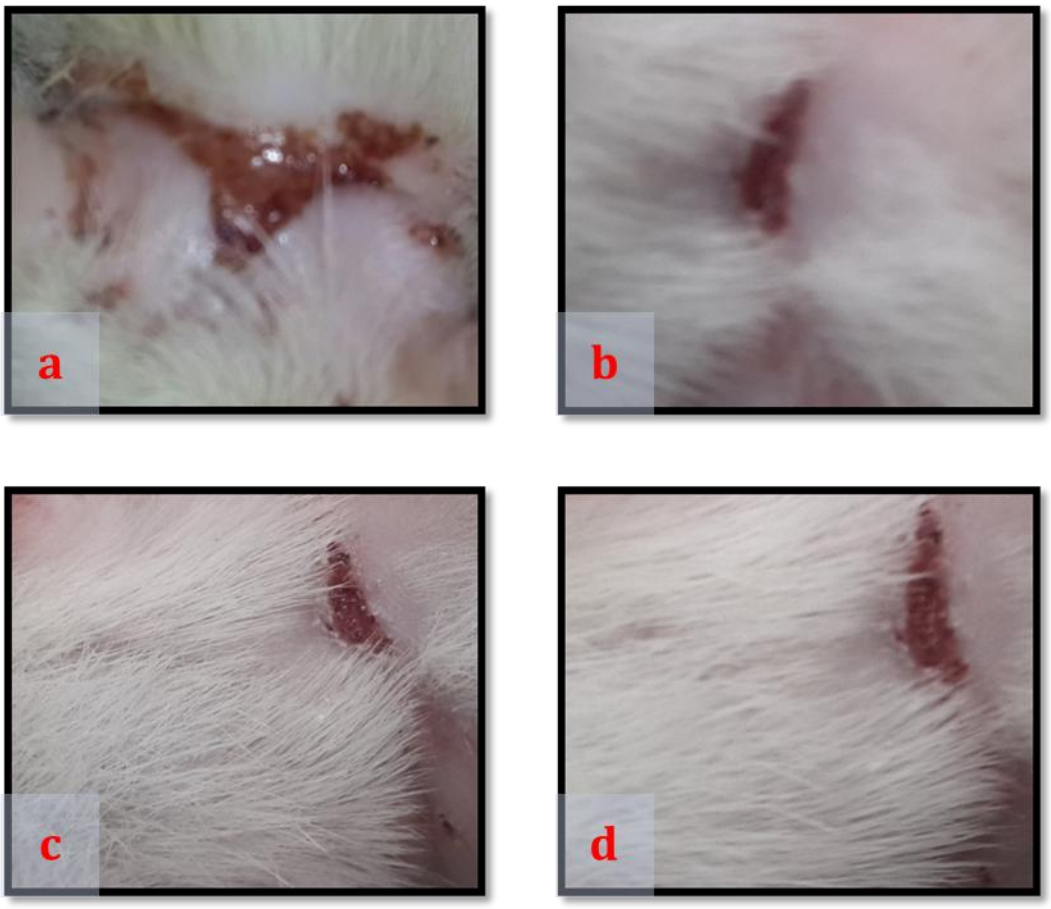


FIG NO 17: Excision wound model on 12th day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

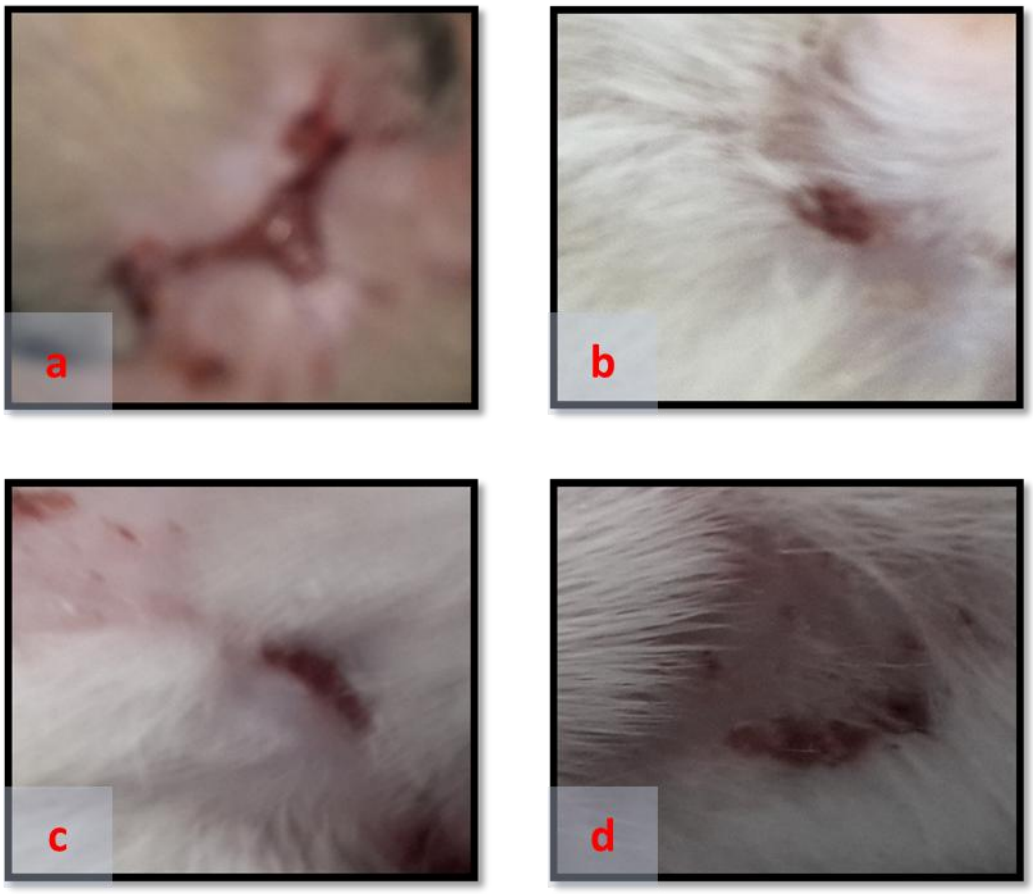


FIG NO 18: Excision wound model on 14th day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

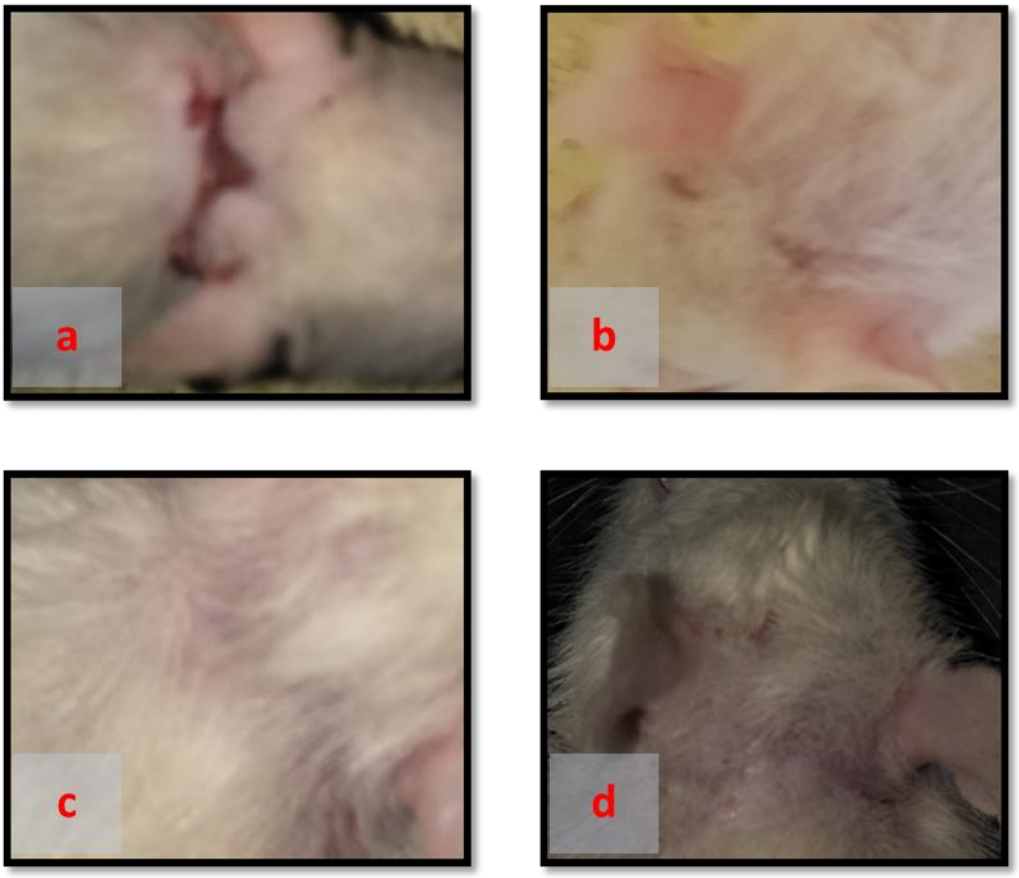


FIG NO 19: Excision wound model on 16th day after wound creation.

a) Control,

b) 2 % w/w of standard drug treated animal,

c) 20 % w/w of Ethanolic extract of *D.patulus* treated animal,

d) 20 % w/w of Chloroform extract of *D.patulus* treated animal.

Chorioallantoic membrane model (CAM)

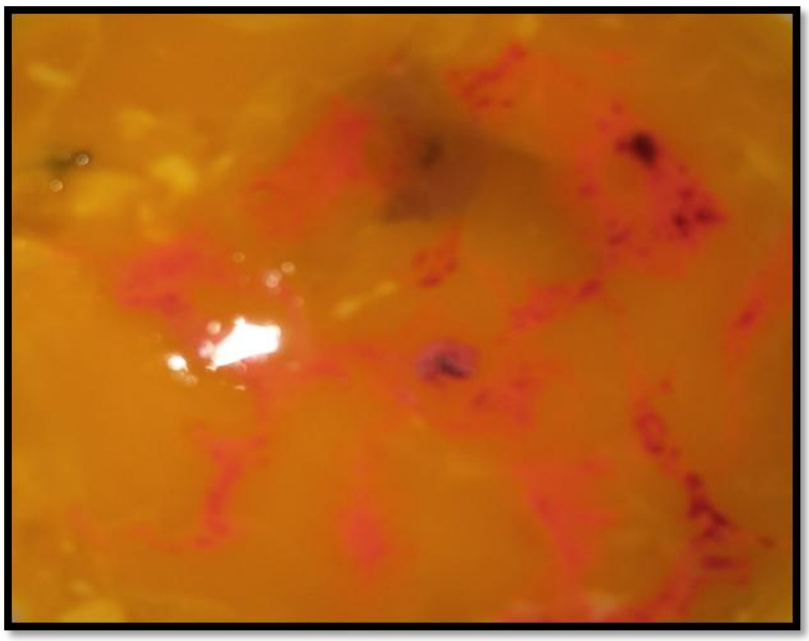


Fig no 20: Treated 500 µg/ml of Ethanolic extract of *D.patulus*.

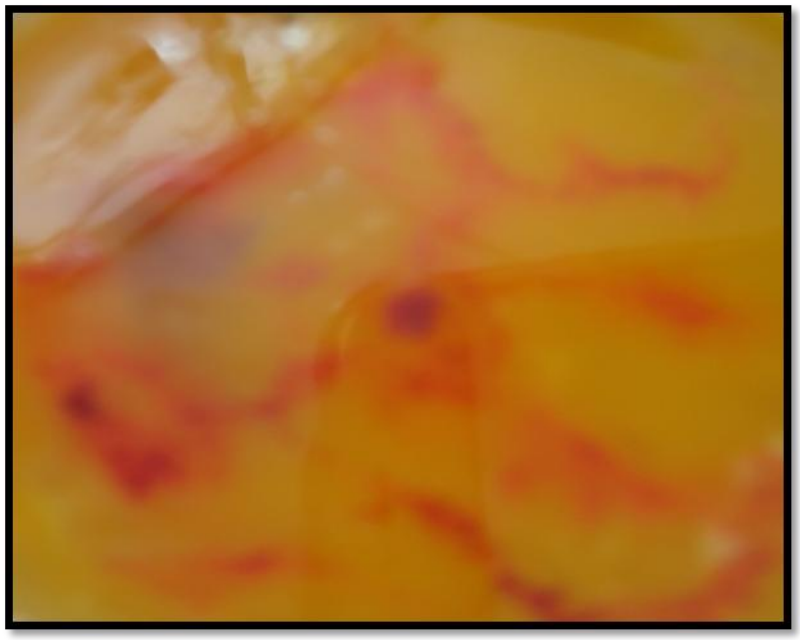


Fig no 21: Treated 500 µg/ml of Chloroform extract of *D.patulus*.

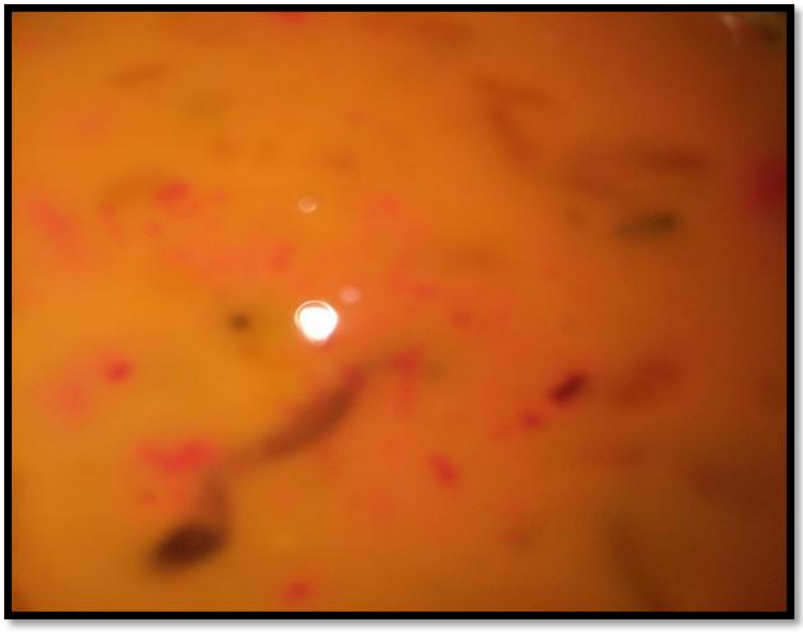


Fig no 22: Positive Control Diclofenac sodium-50 µg/ml.

Fig no 23: Negative Control saline.

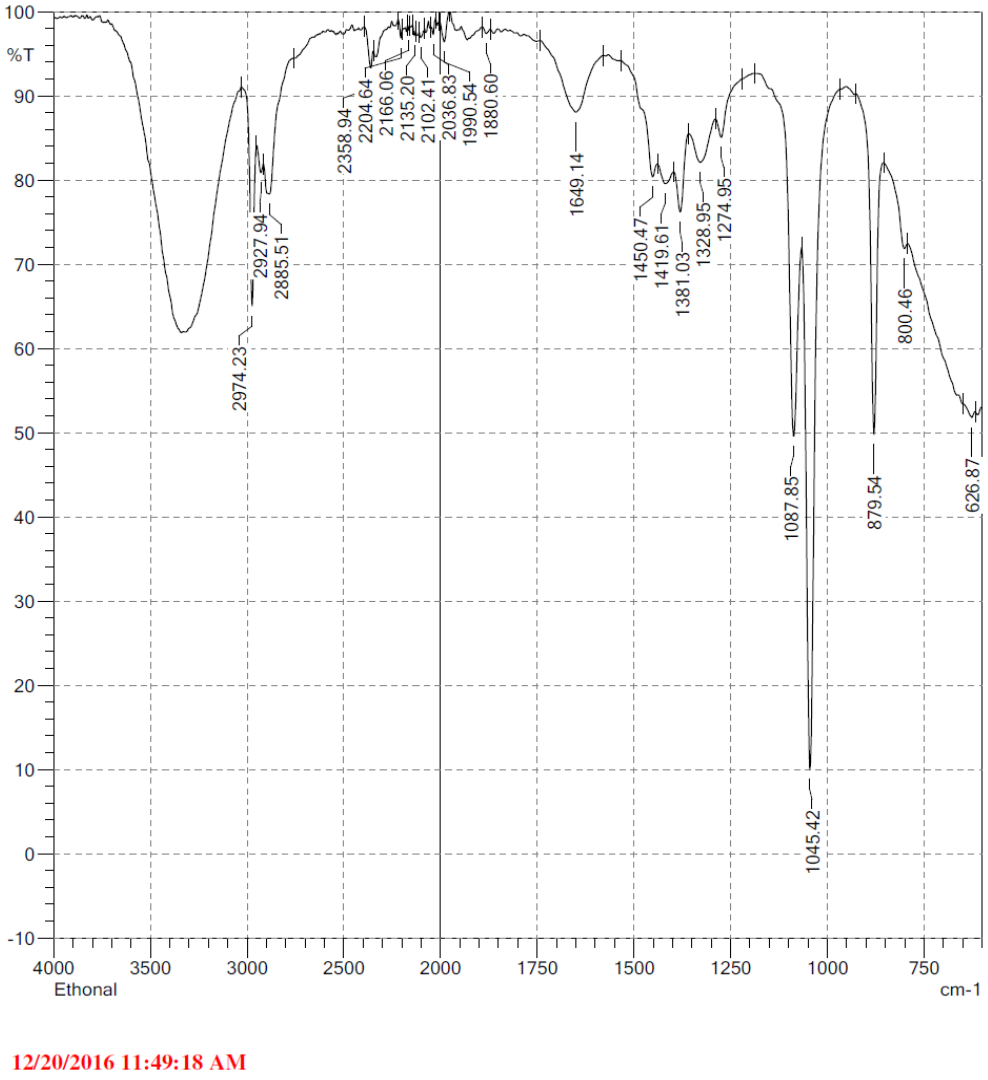


Fig no 24: FT-IR Studies on *D.patulus (Jacq)* Ethanolic extract.

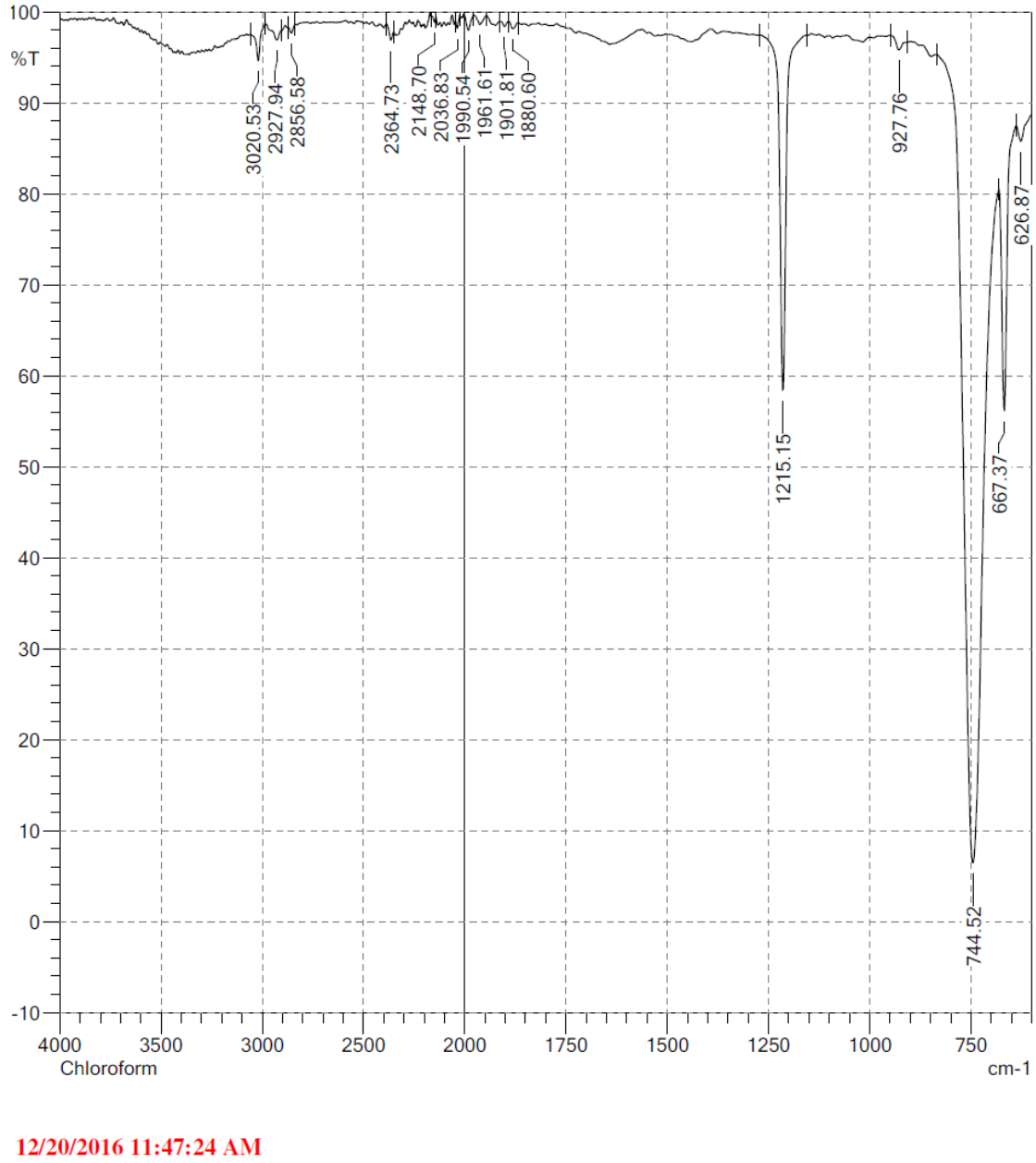


Fig no 25: FT-IR Studies on *D.patulus (Jacq)* Chloroform extract.

Table 3: Effect of *Dipteracanthus patulus* leaf extracts on Aspirin induced ulcer model.

S.No	Treatment	Dose (mg / kg)	Ulcer index	Protection %
1	Control tween 80	5mg / kg	5.57 ± 0.23	---
2.	Ranitidine	30 mg / kg	1.69 ± 0.10**	69. 65 %
3.	Ethanolic extract	100 mg / kg	3.05 ± 0.12**	45.24%
4.	Chloroform extract	100 mg / kg	3.44 ± 0.20	38.24 %

Results are expressed as mean ± SEM from four observations as compared to standard group the one way ANOVA is Graph Pad's software method, (**P< 0.0001) by conventional criteria; this difference is considered to be extremely statistically significant.

Table 4: Effect of *Dipteracanthus patulus* leaf extracts on Ethanol induced ulcer model.

S.No	Treatment	Dose (mg / kg)	Ulcer index	Protection %
1	Control tween 80	5 mg / kg	5.75 ± 0.08	---
2.	Ranitidine	30 mg / kg	1.54 ± 0.13**	73.21%
3.	Ethanolic extract	100 mg / kg	2.72 ± 0.09**	52.69%
4.	Chloroform extract	100 mg / kg	2.97 ± 0.20	48.34%

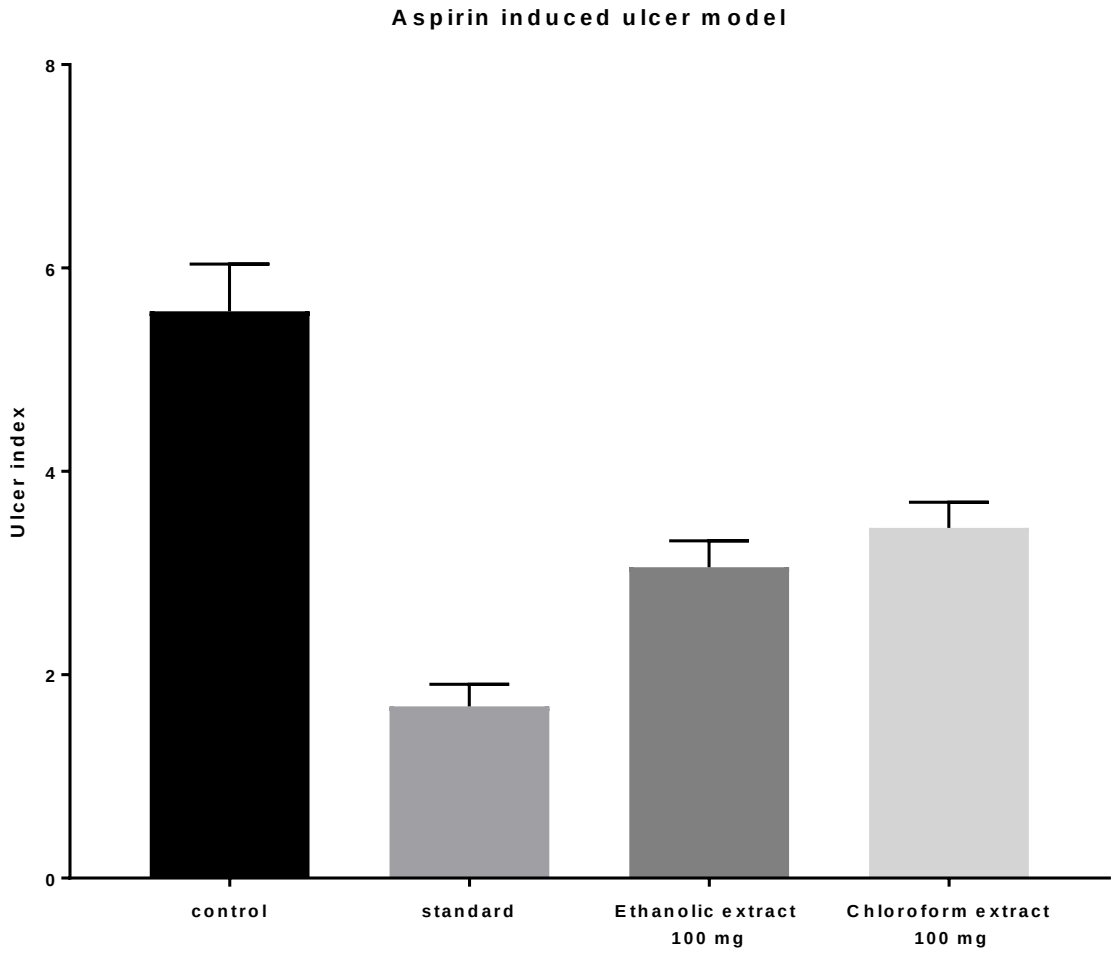
Results are expressed as mean ± SEM from four observations as compared to standard group the one way ANOVA is Graph Pad's software method, (**P< 0.0001) by conventional criteria; this difference is considered to be extremely statistically significant.

Table 5: Effect of *Dipteracanthus patulus* leaf extracts on Excision wound model.

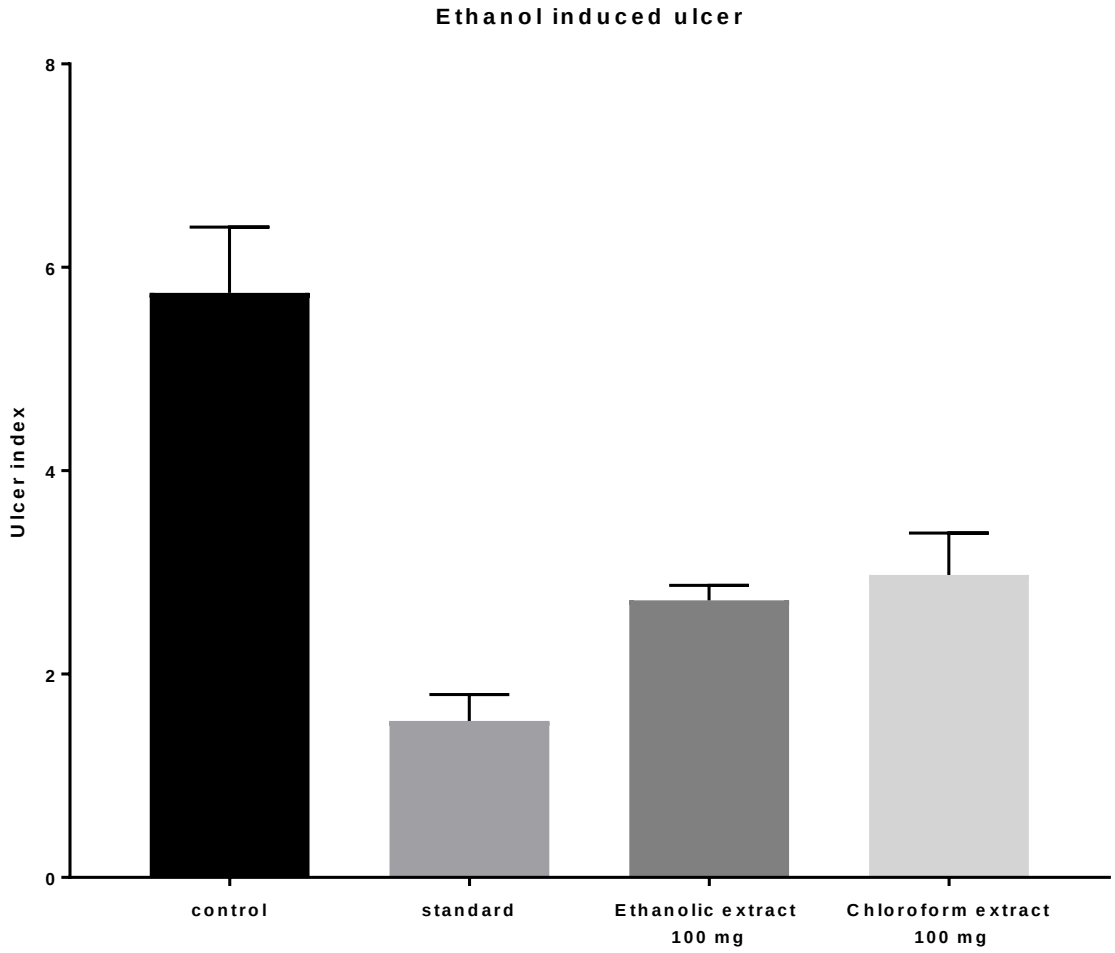
Post wounding days	Wound area (mm^2) (mean ± SEM) and percentage of wound contraction			
	CONTROL	STANDARD DRUG 2%, w/w	ETHANOLIC EXTRACT 20%, w/w	CHLOROFORM EXTRACT 20%, w/w
0	505.33± 11.87	519.90 ± 6.69	505.16 ± 5.02	509.67 ± 5.65
2	476.33 ± 2.08 (5.7%)	452.68 ± 6.09 (12.92%)	451.56 ±15.33 (10.61%)	453.77 ± 7.17 (10.96%)
4	448.54 ± 9.90 (11.23%)	350.64 ±2.81* (32.55%)	348.76 ± 2.34* (30.96%)	350.65 ± 3.85* (31.20%)
6	364.13 ± 4.04 (27.94%)	219.78±1.41** (57.72%)	216.10 ± 2.07* (57.72%)	216.83 ± 1.85 (57.45%)
8	321.31 ± 2.25 (36.41%)	151.93 ± 2.53 (70.77%)	152.56 ± 2.93 (69.79%)	155.01 ± 1.60** (69.58%)
10	250.43 ± 1.51 (50.43%)	94.58 ± 0.92** (81.80%)	95.55 ± 0.95** (81.08%)	93.63 ± 1.65** (81.62%)
12	182.29 ± 3.30 (63.92%)	35.41 ± 0.76** (93.18%)	36.33 ± 1.34** (92.80%)	36. 31 ± 0.90** (92.87%)
14	127.70 ± 1.60 (74.72%)	4.84 ± 0.17** (99.06%)	5.29 ± 0.18** (98.95%)	5.07 ± 0.28** (99.01%)
16	82.82 ± 1.27 (83.61%)	00 (100%)	00 (100%)	00 (100%)

Results are expressed as mean ± SEM from four rats; *p < 0.001, **p <0.0001 vs. standard, one way ANOVA followed by Dunnet's t- test.

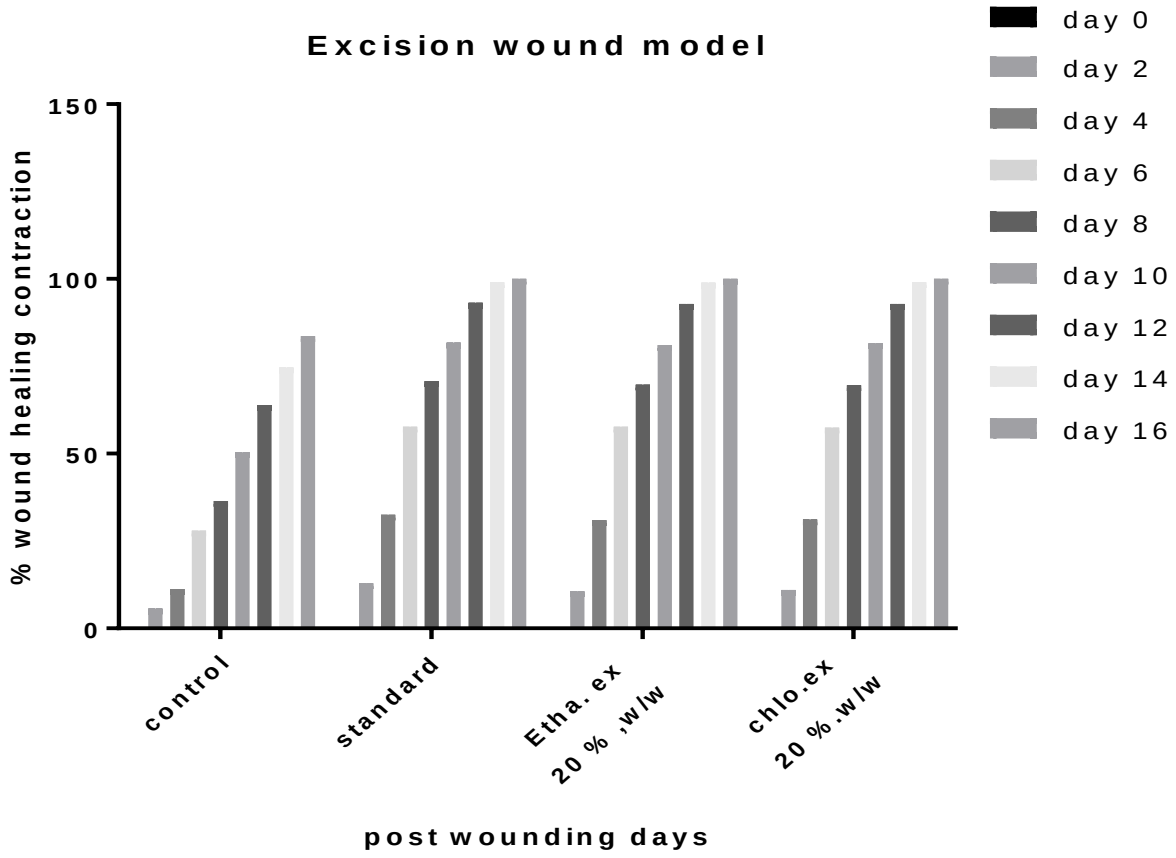
Graph 1: Effect of *Dipteracanthus patulus* leaf extracts on Aspirin induced ulcer model.



Graph 2: Effect of *Dipteracanthus patulus* leaf extracts on Ethanol induced ulcer model.



Graph 3: Effect of *Dipteracanthus patulus* leaf extracts on Excision wound model.



Chapter 6
DISCUSSION

Worldwide, there is a progressive tendency in favor of traditional and integrative health sciences both in research and clinical practice. Similarly both the chloroform, ethanolic extracts were evaluated for their anti-ulcer activity in aspirin and ethanol induced ulcer model in male Wistar rats. Numerous factors are concerned in the ulcerogenesis and gastric mucosal damage induced by different models active in the present study relating, mucosal damage induced by non-steroidal anti-inflammatory drugs and excessive of free radical production. The extracts of the *Dipteracanthus patulus* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of ulcer index as compared to control group signifying its potent cytoprotective effect. It is recommended that *Dipteracanthus patulus* extracts can suppress gastric damage induced by aggressive factors. It is commonly accepted that gastric ulcers result from an imbalance between aggressive factors then the keep the mucosal honesty through endogenous protection mechanisms. The excess gastric acid formation through prostaglandin (PG) increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin in the stomach. Inhibitions of PG synthesis by aspirin concur with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells of the stomach region.

Flavanoids are among the cytoprotective materials for which anti-ulcerogenic efficacy has been widely established. The preliminary phytochemical studies revealed that the presence of flavonoids in ethanolic extract of *Dipteracanthus patulus.* Consequently the possible mechanism of antiulcer action of *Dipteracanthus Paulus* may be due to its flavonoid content. In this study, we observed that *Dipteracanthus patulus provides* significant anti-ulcer activity against gastric ulcers in rats. Both of the extracts produced a significant ($p<0.001$) anti-ulcer activity. The significant increase in the antiulcer activity of *Dipteracanthus patulus (Jacq)* can be credited to the presence of flavanoids, alkaloids, tannins, saponins, glycosides, and steroids. It is suggested that these active compounds would be able to stimulate mucus, bicarbonate and the

prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen.[42]

In NSAIDs-induced gastric ulcer model, the ulcers were induced in rats by the administration of Aspirin (500mg/ kg p.o). There will be eradication of the prostaglandins from the stomach mucosal region, which may cause ulceration, such as the prostaglandins need the major role in the gastric mucosa production. Hence, the purging of prostaglandins it reduces the protective mucosal secretion and increases acid secretion, thereby leading to ulceration in the stomach. In the present study, in gastric ulcer model induced by Aspirin (500mg/kg p.o) in rats the values of ulcer index were reduced in the treated group by *D. patulus* ethanol extract 100mg/kg (3.05±0.12) and chloroform extract 100mg/kg (3.44±0.20) there was a significant reduction in the ulcer index (**P<0.0001) as standard compared to control group.[43]

Ethanol-induced ulcers major in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4),mast cell secretory products, and re active oxygen species resulting in the damage of rat gastric mucosa. In ethanol model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells leading to the release of the vasoactive mediators as well as histamine, therefore producing damage to gastric mucosa. Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin.

In the present study, in gastric ulcer model induced by Ethanol (1ml/kg p.o) in rats the values of ulcer index were reduced in the treated group *D. patulus* ethanolic extract 100 mg/kg (2.72±0.09) and chloroform extract 100mg/kg (2.97±0.20), there was a significant reduction in the ulcer index (**P<0.0001) as standard compared to control group.[44]

The repair of wounds involves different phases including contraction, formation of granulation tissue. The biological response regulating the body is own cellular defense mechanisms contributes to the wound and its repair. Topical application of *D. patulus* leaf ethanol and chloroform extract 20 %w/w ointment at wound site in excision wound healing model produced significant (**p <0.0001) wound healing activity. Treated excision wounds showed an increased rate of contraction, leading to closer healing as confirmed by the increased healed area when compared to the control group. Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state.[45]

In Chorioallantoic membrane Assay model, Angiogenesis is vital in normal processes of the development of blood vessel of embryo, formation of corpus luteum and wound healing.[46]

Angiogenesis during wound repair helps the two fold function of providing the nutrients required by the healing tissue and contributing to structural repair through the formation of granulation tissue.[47]

D. patulus both extract stimulated angiogenesis as proved by CAM model showed a faster wound contraction. By comparing both the extracts, ethanolic extract show better activity than the chloroform extract.

Chapter 7

CONCLUSION

In the conclusion, the Chloroform and ethanolic extract of *Dipteracanthus patulus* leaf that potentiated the anti-ulcer activity and wound healing activity. Both extract could be mainly due to the modulation of defensive factors through an development of gastrointestinal cytoprotection and partly due to suppression of gastric juice. Further *Dipteracanthus patulus* leaf extracts was found to possess wound healing activity on different phases of wound healing, including wound contraction and angiogenesis. In this present study, used parameters on exposure to Aspirin and Ethanol induced ulcer model in rats and Excision wound model in rats and chorioallantoic membrane assay. The results obtained from these experimental models clearly confirmed the ethanolic and chloroform extract of *Dipteracanthus patulus* Leaf possessed Anti-ulcer activity and wound healing activity.

Therefore further studies will be focused on the preliminary phytochemical studies responsible for the observed active constituents have to be isolated and identified.

Chapter 8

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